Stephen R. Smerek (SBN: 208343)
WINSTON & STRAWN LLP
333 South Grand Avenue, 38th Floor
Los Angeles, CA 90071-1543
Telephone: 213-615-1700
Facsimile: 213-615-1750
Email: ssmerek@winston.com

George C. Lombardi
Raymond C. Perkins
Stephanie S. McCallum
Kevin E. Warner
WINSTON & STRAWN LLP
35 West Wacker Drive
Chicago, IL 60601
Telephone: 312-558-5600
Facsimile: 312-558-5700
Email: glombardi@winston.com

Attorneys for Plaintiff
ABBOTT LABORATORIES

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

ABBOTT LABORATORIES, Plaintiff, v. SCANTIBODIES LABORATORY, INC., Defendant.	Case No. 08 CV 1525 H (BLM) **NOTICE OF APPLICATION AND EX PARTE APPLICATION FOR AN ORDER SHORTENING TIME ON ABBOTT LABORATORIES' NOTICE OF MOTION AND MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON SCANTIBODIES LABORATORY, INC; MEMORANDUM OF POINTS AND AUTHORITIES AND DECLARATION OF KEVIN E. WARNER IN SUPPORT THEREOF** Date: TBD Time: TBD Courtroom: 13 Judge: The Hon. Marilyn L. Huff Magistrate: The Hon. Barbara Lynn Major

TO THIS HONORABLE COURT, ALL INTERESTED PARTIES, AND THEIR COUNSEL OF RECORD:

PLEASE TAKE NOTICE, that Plaintiff Abbott Laboratories ("Abbott") will and hereby does apply *ex parte* for an order shortening time on Abbott Laboratories' Notice of Motion and Motion to Compel Compliance with Subpoena on Scantibodies Laboratory, Inc. Pursuant to Rule 45(c)(2)(B) (the "Application").

This Application is made upon good cause existing. Specifically, the fact discovery cutoff in the underlying matter, pending in the Northern District of Illinois, is September 26, 2008, and Scantibodies Laboratory, Inc. ("Scantibodies") has failed to produce documents and present a date for deposition of any corporate designees in response to a Subpoena served on April 25, 2008, and has failed to permit inspection of its premises pursuant to a Subpoena served on August 4, 2008. Abbott will be prejudiced and harmed in connection with the underlying matter if Abbott cannot depose and obtain responsive documents from Scantibodies, and inspect the relevant premises of Scantibodies, before September 26, 2008.

This Application is made pursuant to Local Rule 7.1, and is based on (a) this Notice; (b) the attached Memorandum of Points and Authorities; (c) the Declaration of Kevin E. Warner; (d) the [Proposed] Order; and (e) the arguments of counsel at the time of the hearing on the Application, if any such hearing is required by the Court.

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Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

Dated: August 22, 2008

WINSTON & STRAWN LLP

By: /s/Stephen R. Smerek

Stephen R. Smerek (SBN: 208343)
333 South Grand Avenue, 38th Floor
Los Angeles, CA 90071-1543
Telephone: 213-615-1700
Facsimile: 213-615-1750
Email: ssmerek@winston.com

and

George C. Lombardi
Raymond C. Perkins
Stephanie S. McCallum
Kevin E. Warner
35 West Wacker Drive
Chicago, IL 60601
Telephone: 312-558-5600
Facsimile: 312-558-5700
Email: glombardi@winston.com

Attorneys for Plaintiff
ABBOTT LABORATORIES

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

MEMORANDUM OF POINTS AND AUTHORITIES

Pursuant to Local Rule 7.1, Plaintiff Abbott Laboratories ("Abbott") files this memorandum of points and authorities in support of its *ex parte* application for an order shortening time on Abbott's Motion to Compel Compliance with Subpoena on Scantibodies Laboratory, Inc. (the "Motion"). The Motion is annexed hereto as Exhibit 1.

I. BACKGROUND

The fact discovery cut-off for the underlying action, a patent infringement case pending in the Northern District of Illinois, is September 26, 2008. (Declaration of Kevin E. Warner ¶ 2.) The patents-in-suit are owned by SurModics, Inc. (a co-plaintiff in the underlying action) and exclusively licensed to Plaintiff Abbott. (Decl. ¶ 3.)

Church & Dwight Co., Inc., the defendant in the underlying case, markets several tests for detecting pregnancy and ovulation under labels such as First Response and Answer. (Decl. ¶ 4.) Abbott and SurModics allege that those products infringe certain claims of the patents-in-suit. (*Id.*) Many of the accused products are manufactured in California by Scantibodies Laboratory, Inc. ("Scantibodies"). (*Id.*)

Scantibodies, as the manufacturer of the accused products, is an important non-party witness. Scantibodies has in its possession, custody and control a broad array of documents related to Abbott's allegations of infringement. (Decl. ¶ 5.) Further, Abbott believes that an inspection of the manufacturing process at Scantibodies will also reveal evidence relevant to the claims of infringement. (*Id.*)

In light of the magnitude of Scantibodies' involvement in the manufacture of the accused products marketed by Church & Dwight, Abbott served a subpoena on Scantibodies on April 25, 2008. (Decl. ¶ 6; Mot. Exh. 2.) In the subpoena, Abbott requested the production of various categories of documents and the designation of one or more witnesses to testify at a deposition on behalf of Scantibodies pursuant to Federal Rule 30(b)(6). (*Id.*) Abbott has also served a subpoena requesting entry on Scantibodies' manufacturing premises

to photograph and/or videotape the manufacturing process of the accused products ultimately sold to consumers by Church & Dwight. (Decl. ¶ 6; Mot. Exh. 10.)

On June 23 and July 21, 2008, Abbott requested that Scantibodies produce the subpoenaed documents and the names and dates of availability of its corporate designees for deposition. (Decl. ¶ 7; *see* Mot. Exhs. 4-5.) Abbott also attempted to schedule a formal "meet and confer" to discuss any issues relating to the subpoena. (Decl. ¶ 7; Mot. Exh. 5.) In July and August 2008, counsel for Abbott and Scantibodies discussed what was required of Scantibodies in order to meet its obligations under the Federal Rules of Civil Procedure with respect to the subpoena. (Decl. ¶ 7; *see* Mot Exhs. 6-9.) After those communications, Scantibodies asserted its view that it need only produce documents responsive to one of the eighteen categories of documents contained in the subpoena. (Decl. ¶ 7; Mot. Exh. 6.) After unsuccessful attempts by Abbott's counsel to reach counsel for Scantibodies by phone after July 24, counsel for Abbott provided a detailed explanation clarifying its position in writing on August 4. (Decl. ¶ 7; Mot. Exh. 7.) Scantibodies responded in writing on August 10, reasserting its position that its obligations for discovery were far more limited than what the subpoena actually requires. (Decl. ¶ 7; Mot. Exh. 8.) The following day, Abbott again provided a detailed clarification of what documents were required to meet Scantibodies' discovery obligations, and again sought dates on which one or more corporate representatives of Scantibodies could be deposed as requested in the subpoena. (Decl. ¶ 7; Mot. Exh. 9.) Scantibodies has yet to produce any responsive documents or designate dates for its 30(b)(6) witness's availability. (Decl. ¶ 7.)

Rather than file an immediate motion following service of the subpoena and Scantibodies' first formal objections and responses, Abbott continued discovery in Illinois with the anticipation that Church & Dwight would produce the documents that Abbott requested from Scantibodies. (Decl. ¶ 8.) As soon as it became apparent that Church & Dwight did not have all relevant documents in its possession, Abbott filed its Motion. (*Id.*) With an approaching September 26 discovery deadline in the underlying patent dispute, and

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333 South Grand Avenue
Los Angeles, CA 90071-1543

with Scantibodies' refusal to produce the vast majority of unquestionably relevant discovery requested by the subpoena, Abbott requested that the Court enter an Order compelling Scantibodies to produce all responsive documents by August 28, 2008, and to produce a witness for deposition no later than September 12, 2008. (Decl. ¶ 8; *see* Mot. at 10.)

Abbott has also served a second subpoena on Scantibodies, as noted above, that includes a notice of inspection of Scantibodies' facilities. (Decl. ¶ 9; Mot. Exh. 10.) The requested inspection will provide evidence relevant to the structure of the accused products and the method of their manufacture, all of which is relevant to Abbott's claims of infringement. (Decl. ¶ 9.) Scantibodies informed Abbott on August 18, 2008, that it would not permit the requested inspection. (*Id.*) Given the fact that discovery obtained during the inspection may be relevant to the deposition of Scantibodies and the disputed infringement issues in the underlying case, Abbott requested that the Court set a date for inspection immediately preceding the scheduled deposition. (*Id*; *see* Mot. at 10.)

The filed Motion was served on Wednesday, August 20, 2008. (Decl. ¶ 10; *see* Exh. 2.) Therefore, under Local Rule 7.1(e), the earliest date for a hearing on the Motion would be 28 days following the first Monday after August 20, which is September 22, 2008. Furthermore, even assuming that the Court decides to rule on the Motion on that earliest possible hearing date, only four calendar days will remain for the production of all documents, inspection of the facilities, as well as the deposition. (Decl. ¶ 10.)

II. DISCUSSION

Federal Rule of Civil Procedure 6(d) authorizes the Court to allow shorter times between the filing and service of motion papers and the hearing thereon for good cause shown. Good cause exists to brief and argue the Motion on a shortened schedule because Abbott will be prejudiced if it is denied the requested discovery. Specifically, Abbott currently has until September 26, 2008 to complete fact discovery in the underlying matter, including fact discovery to be obtained from all third parties. Abbott may not be able to obtain all of the necessary discovery it seeks from Scantibodies even under the swiftest

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333 South Grand Avenue
Los Angeles, CA 90071-1543

briefing and hearing schedule provided for by the Local Rules. The earliest possible hearing date on the Motion is September 22, 2008, four days before the close of fact discovery. That brief time frame may not allow for complete document production, an inspection of Scantibodies' premises, and a deposition. Yet Abbott's inability to depose and obtain documents from Scantibodies results from no fault of Abbott's; Scantibodies has simply refused to comply with the subpoena. Abbott should not be penalized in the underlying action as a result of Scantibodies' unreasonable and unjustified delay, and its complete refusal to provide nearly all of the requested discovery.

Scantibodies' continued delay and unreasonable objections to compliance with the subpoenas Abbott has served demonstrates a lack of good faith in responding to the subpoena. (*See* Mot. at 4-9.) Given Scantibodies' reassertions of its intent to only produce documents responsive to one of the eighteen categories of requested documents, its inability to provide certain dates for the deposition of one or more corporate designees, and its unwillingness to provide access to relevant manufacturing facilities for inspection – all notwithstanding the unquestionable relevance of the requested discovery to the Plaintiffs' claims in the underlying patent action – judicial intervention is necessary to secure a date for Scantibodies' compliance with the subpoena in advance of the fact discovery cut off in the underlying litigation. Because Abbott must obtain all of this discovery before September 26, 2008, *ex parte* relief is necessary.

III. CONCLUSION

For the foregoing reasons, Abbott respectfully requests that this Court enter an order shortening time, setting (1) the hearing on the Motion for September 4, 2008, or as soon thereafter as the Court deems appropriate; (2) Scantibodies' and Church & Dwight's time to file any opposition to the Motion for August 28, 2008, or as soon thereafter as the Court deems appropriate; and (3) Abbott's time to file a reply for September 2, 2008, or as soon thereafter as the Court deems appropriate.

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Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

Respectfully submitted,

Dated: August 22, 2008

WINSTON & STRAWN LLP

By: /s/Stephen R. Smerek

Stephen R. Smerek
Attorneys for Plaintiff
ABBOTT LABORATORIES
E-mail: ssmerek@winston.com

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

DECLARATION OF KEVIN E. WARNER IN SUPPORT OF EX PARTE APPLICATION FOR ORDER SHORTENING TIME

I, Kevin E. Warner, declare:

1. I am an attorney at law in the State of Illinois associated with Winston & Strawn LLP, counsel for Plaintiff Abbott Laboratories. I have personal knowledge of the facts set forth herein.

2. The fact discovery cut-off for the underlying action, a patent infringement case pending in the Northern District of Illinois, is September 26, 2008.

3. The patents-in-suit are owned by SurModics, Inc. (a co-plaintiff in the underlying action, *see* Mot. Exh. 1) and exclusively licensed to Plaintiff Abbott.

4. Church & Dwight Co., Inc., the defendant in the underlying case, markets several tests for detecting pregnancy and ovulation under labels such as First Response and Answer. Abbott and SurModics allege that those products infringe certain claims of the patents-in-suit. Many of the accused products are manufactured in California by Scantibodies Laboratory, Inc. ("Scantibodies").

5. Scantibodies, as the manufacturer of the accused products, is an important non-party witness. Scantibodies has in its possession, custody and control a broad array of documents related to Abbott's allegations of infringement. Further, Abbott believes that an inspection of the manufacturing process at Scantibodies will also reveal evidence relevant to the claims of infringement.

6. In light of the magnitude of Scantibodies' involvement in the manufacture of the accused products marketed by Church & Dwight, Abbott served a subpoena on Scantibodies on April 25, 2008. (Mot. Exh. 2.) In the subpoena, Abbott requested the production of various categories of documents and the designation of one or more witnesses to testify at a deposition on behalf of Scantibodies pursuant to Federal Rule 30(b)(6). (*Id.*) Abbott has also served a subpoena requesting entry on Scantibodies' manufacturing premises

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

to photograph and/or videotape the manufacturing process of the accused products ultimately sold to consumers by Church & Dwight. (Mot. Exh. 10.)

7. On June 23 and July 21, 2008, Abbott requested that Scantibodies produce the subpoenaed documents and the names and dates of availability of its corporate designees for deposition. (*See* Mot. Exhs. 4-5.) Abbott also attempted to schedule a formal "meet and confer" to discuss any issues relating to the subpoena. (Mot. Exh. 5.) In July and August 2008, counsel for Abbott and Scantibodies discussed what was required of Scantibodies in order to meet its obligations under the Federal Rules of Civil Procedure with respect to the subpoena. (*See* Mot Exhs. 6-9.) After those communications, Scantibodies asserted its view that it need only produce documents responsive to one of the eighteen categories of documents contained in the subpoena. (Mot. Exh. 6.) After unsuccessful attempts by Abbott's counsel to reach counsel for Scantibodies by phone after July 24, counsel for Abbott provided a detailed explanation clarifying its position in writing on August 4. (Mot. Exh. 7.) Scantibodies responded in writing on August 10, reasserting its position that its obligations for discovery were far more limited than what the subpoena actually requires. (Mot. Exh. 8.) The following day, Abbott again provided a detailed clarification of what documents were required to meet Scantibodies' discovery obligations, and again sought dates on which one or more corporate representatives of Scantibodies could be deposed as requested in the subpoena. (Mot. Exh. 9.) Scantibodies has yet to produce any responsive documents or designate dates for its 30(b)(6) witness's availability.

8. Rather than file an immediate motion following service of the subpoena and Scantibodies' first formal objections and responses, Abbott continued discovery in Illinois with the anticipation that Church & Dwight would produce the documents that Abbott requested from Scantibodies. As soon as it became apparent that Church & Dwight did not have all relevant documents in its possession, Abbott filed its Motion, in which Abbott requested that the Court enter an Order compelling Scantibodies to produce all responsive

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

documents by August 28, 2008, and to produce a witness for deposition no later than September 12, 2008. (*See* Mot. at 10.)

9. Abbott has also served a second subpoena on Scantibodies that includes a notice of inspection of Scantibodies' facilities. (Mot. Exh. 10.) The requested inspection will provide evidence relevant to the structure of the accused products and the method of their manufacture, all of which is relevant to Abbott's claims of infringement. Scantibodies informed Abbott on August 18, 2008, that it would not permit the requested inspection. Given the fact that discovery obtained during the inspection may be relevant to the deposition of Scantibodies and the disputed infringement issues in the underlying case, Abbott requested that the Court set a date for inspection immediately preceding the scheduled deposition. (*See* Mot. at 10.)

10. The filed Motion was served on August 20, 2008. (*See* Exh. 2.) Under Local Rule 7.1(e), the earliest date for a hearing on the Motion would be 28 days following the first Monday after August 20, which is September 22, 2008. Furthermore, even assuming that the Court decides to rule on the Motion on that earliest possible hearing date, only four calendar days will remain for the production of all documents, inspection of the facilities, as well as the deposition.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

August 22, 2008

Kevin E. Warner

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

CERTIFICATE OF SERVICE

Abbott Laboratories v. Scantibodies Laboratory, Inc.

I, Stephen R. Smerek, an attorney at Winston & Strawn LLP, of the office located at 333 South Grand Avenue, 38th Floor, Los Angeles, CA 90071-1543, hereby certify under penalty of perjury that on August 22, 2008, that I caused to be served the following documents entitled:

1. NOTICE OF APPLICATION AND EX PARTE APPLICATION FOR AN ORDER SHORTENING TIME ON ABBOTT LABORATORIES' NOTICE OF MOTION AND MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON SCANTIBODIES LABORATORY, INC; MEMORANDUM OF POINTS AND AUTHORITIES AND DECLARATION OF KEVIN E. WARNER IN SUPPORT THEREOF

2. [PROPOSED] ORDER GRANTING ABBOTT LABORATORIES' EX PARTE APPLICATION FOR AN ORDER SHORTENING TIME ON ABBOTT LABORATORIES' MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON SCANTIBODIES LABORATORY, INC. [Per Court's Local Rule Proposed Order emailed [not filed]To The Hon. Barbara Lynn Major at efile_major@casd.uscourts.gov

Said documents were served as indicated below, upon all interested parties at the following addresses:

☒ By Electronic Filing: The above and foregoing document(s) was electronically filed with the Clerk of the Court using the CM/ECF system, which sent notification of such filing to the following

Edward Patrick Swan, Jr. - pswan@luce.com
LUCE, FORWARD, HAMILTON & SCRIPPS LLP
600 West Broadway, Suite 2600
San Diego, CA 92101-3372

Anastasia Heffner - aheffener@usebrinks.com
Glen Belvis - gbelvis@usebrinks.com
BRINKS, HOFER, GILSON & LIONE
NBC Tower, Suite 3600
455 North Cityfront Plaza Drive
Chicago, IL 60611-5599

James H. Shalek - jshalek@proskauer.com
Baldassare Vinti - bvinti@proskauer.com
John Stellabotte - jstellabottr@proskauer.com
Steven Holinstat - sholinstat@proskauer.com
PROSKAUER ROSE LLP
1585 Broadway
New York, NY 10036-8299

Cyrus A. Morton - cmorton@rkmc.com
Trevor J. Foster - tjfoster@rkmc.com
ROBINS, KAPLAN, MILLER & CIRESI
2800 LaSalle Plaza
800 LaSalle Avenue
Minneapolis, MN 55402

DATED: AUGUST 22, 2008

/s/Stephen R. Smerek
Stephen R. Smerek

EXHIBIT 1

FILED

2008 AUG 19 PM 4:34

CLERK US DISTRICT COURT
SOUTHERN DISTRICT OF CALIFORNIA

BY _____ DEPUTY

Stephen R. Smerek (SBN: 208343)
WINSTON & STRAWN LLP
333 South Grand Avenue, 38th Floor
Los Angeles, CA 90071-1543
Telephone: 213-615-1700
Facsimile: 213-615-1750
Email: ssmerek@winston.com

George C. Lombardi
Raymond C. Perkins
Stephanie S. McCallum
Kevin E. Warner
WINSTON & STRAWN LLP
35 West Wacker Drive
Chicago, IL 60601
Telephone: 312-558-5600
Facsimile: 312-558-5700
Email: glombardi@winston.com

Attorneys for Plaintiff
ABBOTT LABORATORIES

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

'08 CV 1525 H BLM

ABBOTT LABORATORIES,

Plaintiff,

v.

SCANTIBODIES LABORATORY, INC.,

Defendant.

Misc. Action No.

Case No. 07 CV 3428 (MFK)
Pending in the Northern District of Illinois

ABBOTT LABORATORIES' NOTICE OF MOTION AND MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES LABORATORY, INC., PURSUANT TO RULE 45(C)(2)(B); MEMORANDUM OF POINTS AND AUTHORITIES AND DECLARATION OF KEVIN E. WARNER IN SUPPORT THEREOF

Date: TBD
Time: TBD
Department: TBD

ORIGINAL

Stephen R. Smerek (SBN: 208343)
WINSTON & STRAWN LLP
333 South Grand Avenue, 38th Floor
Los Angeles, CA 90071-1543
Telephone: 213-615-1700
Facsimile: 213-615-1750
Email: ssmerek@winston.com

George C. Lombardi
Raymond C. Perkins
Stephanie S. McCallum
Kevin E. Warner
WINSTON & STRAWN LLP
35 West Wacker Drive
Chicago, IL 60601
Telephone: 312-558-5600
Facsimile: 312-558-5700
Email: glombardi@winston.com

Attorneys for Plaintiff
ABBOTT LABORATORIES

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

ABBOTT LABORATORIES,

Plaintiff,

v.

SCANTIBODIES LABORATORY, INC.,

Defendant.

Misc. Action No.

Case No. 07 CV 3428 (MFK)
Pending in the Northern District of Illinois

ABBOTT LABORATORIES' NOTICE OF MOTION AND MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES LABORATORY, INC., PURSUANT TO RULE 45(C)(2)(B); MEMORANDUM OF POINTS AND AUTHORITIES AND DECLARATION OF KEVIN E. WARNER IN SUPPORT THEREOF

Date: TBD
Time: TBD
Department: TBD

PLEASE TAKE NOTICE that on _____, 2008, at 9:00 a.m., or as soon thereafter as this matter may be heard in the Courtroom of _____ of the above-entitled Court, located at 880 Front Street, San Diego, CA 92101, Plaintiff Abbott Laboratories will and hereby does move, pursuant to Federal Rules of Civil Procedure, Rule 45(c)(2)(B), for an Order Compelling Compliance with Subpoena on Third Party Scantibodies Laboratory, Inc. This Motion is brought pursuant to Rule 45(c)(2)(B) of the Federal Rules of Civil Procedure on the grounds that:

- Scantibodies has failed to produce responsive documents or provide dates for corporate designees to provide deposition testimony responsive to the subpoena; and
- Scantibodies has failed to permit inspection of its facilities as requested pursuant to subpoena.

This Motion is based on this Notice of Motion and Motion, the attached Memorandum of Points and Authorities and Declaration of Kevin E. Warner, all pleadings and papers on file herein, and upon such further oral and documentary evidence as may be presented at or before the hearing on this Motion.

CERTIFICATE OF COMPLIANCE WITH LOCAL RULE 26.1

Pursuant to Local Rule 26.1, Abbott hereby certifies that counsel for Abbott has correponded with counsel for Scantibodies both by phone and in writing regarding the disputed issues, and has also attempted to schedule a formal "meet and confer" to address these disputed issues with counsel for Scantibodies on July 21, 2008, that it made further unsuccessful attempts to reach Scantibodies' counsel by telephone between July 24, 2008, and August 18, 2008, and that such attempts to confer by telephone were unreturned by Scantibodies' counsel. *See* Declaration of Kevin E. Warner, attached to this motion.

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Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

Dated: August 19, 2008

WINSTON & STRAWN LLP

By: _____

Stephen R. Smerek (SBN: 208343)
333 South Grand Avenue, 38th Floor
Los Angeles, CA 90071-1543
Telephone: 213-615-1700
Facsimile: 213-615-1750
Email: ssmerek@winston.com

and

George C. Lombardi
Raymond C. Perkins
Stephanie S. McCallum
Kevin E. Warner
35 West Wacker Drive
Chicago, IL 60601
Telephone: 312-558-5600
Facsimile: 312-558-5700
Email: glombardi@winston.com

Attorneys for Plaintiff
ABBOTT LABORATORIES

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

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Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

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Los Angeles, CA 90071-1543

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333 South Grand Avenue
Los Angeles, CA 90071-1543

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

MEMORANDUM OF POINTS AND AUTHORITIES

I. INTRODUCTION

In over three months since being served with a subpoena for documents and deposition in a patent case pending in the Northern District of Illinois, Scantibodies Laboratory, Inc. – the manufacturer of the accused products – has failed to produce a single document or present dates for any corporate designees to provide deposition testimony responsive to the subpoena. The close of fact discovery in the underlying dispute is currently September 26, 2008. Given that approaching deadline, Plaintiff Abbott Laboratories ("Abbott") cannot afford any further delays by Scantibodies in obtaining necessary discovery. Abbott therefore submits this Memorandum of Points and Authorities in Support of its Motion to Compel Compliance with Subpoena on Third Party Scantibodies Laboratory, Inc. ("Scantibodies"), and to compel production of documents, a witness for deposition, and an inspection of Scantibodies' premises, pursuant to Rules 30, 34 and 45(c)(2)(B) of the Federal Rules of Civil Procedure.

Abbott respectfully requests by this motion that the Court enforce Abbott's subpoena by compelling Scantibodies to produce all non-privileged documents responsive to Abbott's subpoena by August 29, 2008, produce a witness for deposition by no later than September 12, 2008, and permit Abbott to inspect Scantibodies' facilities on the day immediately preceding the scheduled deposition.

II. FACTUAL BACKGROUND

This is a patent infringement case involving two patents owned by SurModics, Inc. (a co-plaintiff in the underlying action) and exclusively licensed to Plaintiff Abbott. The complaint, filed in the Northern District of Illinois, is annexed hereto as Exhibit 1. (Declaration of Kevin E. Warner ¶ 2; Exh.1.) The patents-in-suit, U.S. Patent Nos. 5,654,162 and 6,020,147, (Decl. ¶ 3; Exh. 1 at internal Exhibits A and B), cover various types of immunoassays used to detect the presence of substances in sample fluids.

One common embodiment of this technology is home-use pregnancy test kits and tests for the prediction of ovulation that are found, for instance, in local pharmacies. (Decl. ¶ 3.) In the case of pregnancy test kits, the immunoassays are designed to detect the presence of a hormone called "hCG" in female urine samples, the presence of which indicates that the woman is pregnant. (*Id.* at ¶ 4.) The tests often operate by using proteins called "antibodies" that naturally bind to hCG, and then detecting labels or tags – such as small colored particles – that are attached to the antibodies. (*Id.*) The creation and detection of a visible, colored signal at a test area on the device indicates that the antibodies have bound with hCG, that hCG is present in the sample, and that the test user is pregnant. (*Id.*) The absence of any color development generally means the labeled-antibody did not detect hCG, therefore indicating that the user is not pregnant. (*Id.*) Tests to predict when a woman is ovulating operate on the same principle, except that the antibodies in those products detect a hormone indicative of ovulation called leuteinzing hormone, or "LH." (*Id.*)

Church & Dwight Co., Inc., the defendant in the underlying case, markets several tests for detecting pregnancy and ovulation under labels such as First Response and Answer. (Decl. ¶ 5.) Abbott and SurModics allege that those products infringe certain claims of the '162 and '147 patents. (*Id.*) Discovery has revealed, however, that Church & Dwight is not itself the manufacturer of the accused products. (Decl. ¶ 6.) Many of the accused products are manufactured in California by Scantibodies. (*Id.*)

Scantibodies, as the manufacturer of the accused products, has important documents related to Abbott's allegations of infringement. (Decl. ¶ 7.) Abbott believes, for instance, that Scantibodies has documents related to the types and properties of the antibodies used in the accused products. (*Id.*) The characterization and properties of those antibodies are relevant to Abbott's infringement claims. (*Id.*) As another example, Scantibodies also has manufacturing documents and specifications related to the assembly of the accused devices which documents also would be relevant to Abbott's infringement claims. (*Id.*) Abbott believes Scantibodies also has agreements with Church & Dwight and communications with

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

Church & Dwight that bear on several issues in the case, from Church & Dwight's infringement of the patents-in-suit to the damages owed to Abbott and SurModics. (*Id.*) Further, Abbott believes that an inspection of the manufacturing process at Scantibodies will also reveal evidence relevant to the claims of infringement. (*Id.*)

In light of the magnitude of Scantibodies' involvement in the manufacture of the accused products marketed by Church & Dwight, Abbott served a subpoena on Scantibodies on April 25, 2008. (Decl. ¶ 8; Exh. 2.) In the subpoena, Abbott requested the production of various categories of documents and the designation of one or more witnesses to testify at a deposition on behalf of Scantibodies pursuant to Federal Rule 30(b)(6). (*Id.*) Schedule B of the subpoena contains the categories of documents and things Abbott seeks from Scantibodies. (*Id.*) All categories are directly related to the diagnostic products manufactured by Scantibodies for C&D that are at issue in the underlying litigation, or to Scantibodies' relationship with C&D. (Decl. ¶ 8.)

Scantibodies first responded to the subpoena by submitting a formal set of boilerplate objections, but not agreeing to produce *any* documents. (Decl. ¶ 9; Exh. 3.) Instead, Scantibodies hid behind the claim that it should not be "put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to" Abbott's requests "that [Defendant Church & Dwight] might have in its possession, custody or control." (*E.g.*, Exh. 3 at 3.) Scantibodies provided no argument in its objections as to why any one of Abbott's document requests or deposition topics was irrelevant to the underlying litigation or, at the very least, not calculated to lead to the discovery of admissible evidence. (*Id.*)

Following receipt of Scantibodies' objections on May 20, 2008, and in the interests of avoiding conflict between the parties, Kevin Warner, counsel for Abbott, contacted and spoke with counsel for Scantibodies by phone in June 2008 in an effort to discuss the nature of Abbott's requests for documents and deposition, and to provide further insight into the types of documents Abbott believed that Scantibodies, and not Church & Dwight, would

Winston & Strawn LLP
333 South Grand Avenue
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have in its possession. (Decl. ¶ 10.) Abbott's counsel identified documents concerning the testing of the antibodies in the accused products, samples of the antibodies used in the accused products, certain manufacturing documents and certain communications about Church & Dwight and the accused products, as general categories of documents that were likely only in the possession of Scantibodies. (*Id.*) Abbott's counsel indicated that it would welcome production of those documents, and then consider whether Abbott would be in a position to forego further document discovery from Scantibodies. (*Id.*) At no time during that conversation or at any time thereafter did counsel for Abbott indicate that it was withdrawing any requests for documents contained in the subpoena or withdrawing its request for a deposition of Scantibodies on the topics listed in the subpoena. (*Id.*)

Abbott was hopeful that it would soon receive a positive response from Scantibodies, but none came. (Decl. ¶ 11.) Abbott called Scantibodies again in June 2008, but after receiving no response to the voice mail it left for Scantibodies' counsel seeking compliance with the subpoena, counsel for Abbott wrote to Scantibodies' counsel on June 23, 2008, again requesting both the production of responsive documents and the designation of a witness. (*Id*; Exh. 4.) Scantibodies ignored both that voice mail and the e-mail of June 23 for nearly a month. (Decl. ¶ 11.) Still hopeful about avoiding the need to seek this Court's assistance in obtaining discovery, Abbott again wrote to Scantibodies on July 21, 2008, this time requesting a "meet and confer" should Scantibodies' response be less than full compliance with the subpoena. (Decl. ¶ 12; Exh. 5.)

Scantibodies delayed providing any material responsive to Abbott's subpoena for nearly three months after receiving the subpoena. And when it finally did respond, it was with more of the same delay tactics. (Decl. ¶ 13.) Scantibodies responded on July 24, 2008, that it would produce documents responsive to ***one*** of the ***eighteen*** categories of documents contained in Abbott's subpoena, and only if Abbott would provide what amounts to an unreasonable time limitation on that request. (*Id*; Exh. 6.) Moreover, Scantibodies did not

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mention the possibility of a deposition, mentioning neither who Scantibodies' Rule 30(b)(6) designee(s) would be nor providing a date for deposition. (*Id.*)

Abbott made several attempts to call Scantibodies' counsel following that e-mail to address Scantibodies' objection regarding limiting any document production to specified time periods. (Decl. ¶ 14.) As Abbott then explained on August 4, 2008, no time limit could be placed on documents relevant to any testing or evaluation of the antibodies used in the accused products. (*Id*; Exh. 7.) Abbott further explained:

> Documents concerning the antibodies used in any accused C&D Test Kit are relevant, regardless of the date of their creation. For instance, documents concerning the properties and characteristics of the antibodies used in the accused Test Kits are relevant to the extent they were created during the period of SLI's relationship with Church & Dwight. Such documents that relate to the same antibodies and/or cell lines are not irrelevant simply because they may pre-date that relationship.

(*Id.*)

Critically, Abbott also corrected Scantibodies' suggestion that producing documents responsive to a single category concerning documents that report testing and evaluation of the antibodies at issue would suffice to meet Scantibodies' obligations under the subpoena. Abbott made clear that it was "not…withdrawing any or all of its other document requests. To the extent [Scantibodies] has documents responsive to those categories that have not been produced in this litigation by Church & Dwight, we request that you produce those documents as well." (Decl. ¶ 15; Exh. 7) Abbott thus requested full compliance with both the document and deposition requests contained in the subpoena. (*Id.*)

Scantibodies again responded with more delay, betraying a lack of good faith. It maintained its decision to produce documents responsive to only ***one of eighteen*** categories in the subpoena, and agreed only to produce such documents dating back an arbitrary two years based on the alleged two year "shelf-life" of the antibodies that may have been

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analyzed by Scantibodies. (Decl. ¶ 17; Exh. 8.) That "shelf-life" argument bears no relationship to the relevance of a particular document, however. As Abbott stated in response, "A document that characterizes an antibody prior to the expiration of its 'shelf-life' does not become irrelevant or of less importance at any time thereafter simply because the antibody itself may no longer be active." (Decl. ¶ 17; Exh. 9.) Abbott also again explained that documents responsive to only one request would not satisfy Scantibodies' obligations pursuant to Federal Rule 45. (*Id.*)

Scantibodies has never moved this Court to quash the subpoena or issue a protective order, (Decl. ¶ 18), almost certainly because it knows that all of the narrowly crafted discovery requests are calculated to lead to the discovery of admissible evidence. With an approaching, September 26 discovery deadline in the underlying patent dispute, and with Scantibodies' reluctant willingness to produce only a small fraction of responsive documents, Abbott has been left with no reasonable choice but to request this Court's assistance in obtaining necessary discovery. Abbott therefore respectfully requests that this Court enter an Order compelling Scantibodies to produce all responsive documents by August 28, 2008, and to produce a witness for deposition no later than September 12, 2008.

Abbott has also served a second subpoena on Scantibodies including a notice of inspection of Scantibodies' facilities. (Decl. ¶ 19; Exh. 10.) That notice seeks entry of Scantibodies' manufacturing facilities to photograph or videotape the actual manufacturing process of the accused products. That discovery will provide evidence relevant to the structure of the products and the method of their manufacture, all of which is relevant to Abbott's and SurModics' claims of infringement. Scantibodies informed Abbott on August 18, 2008, that it would not permit the requested inspection. (Decl. ¶ 19.) Thus, given the fact that discovery obtained during the inspection may be relevant to the deposition of Scantibodies and the disputed infringement issues in the underlying case, Abbott respectfully requests that this Court set a date for inspection immediately preceding the scheduled deposition.

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III. ARGUMENT

The fundamental precept that the public has a right to everyone's evidence is embodied in Rule 26 and Rule 45 of the Federal Rules of Civil Procedure. *See United States v. Bryan*, 339 U.S. 323, 331 (1950); *Jaffee v. Redmond*, 518 U.S. 1, 9 n.8 (1996), Under Rule 26(b)(1), discovery may be had as to any non-privileged material which is relevant to a claim or defense of any party and, under Rule 45, the scope of materials obtainable by subpoena is as broad as that permitted by Rule 26. *Graham v. Casey's General Stores*, 206 F.R.D. 251, 253 (S.D. Ind. 2003). The party objecting to a subpoena bears the heavy burden of proving that the materials are irrelevant, privileged or that compliance would constitute an undue burden. *Anderson v. Abercrombie and Fitch Stores, Inc.*, No. 06cv991-WQH, 2007 U.S. Dist. LEXIS 47795, at *4-6 (S.D. Cal. July 2, 2007) (establishing that the party making a motion to object, modify, or quash a subpoena bears the burden of persuasion under Rule 45); *see also Irons v. Kareski,* 74 F.3d 1262, 1264 (D.C. Cir. 1996); *Casey's General Stores, supra* at 254 *and cases cited therein*; *Wright, Miller & Marcus, Federal Practice and Procedure:* Civil 2d § 2459 (1994).

The objecting party cannot meet this heavy burden with no or mere conclusory assertions. *See generally Collins v. JC Penney Life Ins. Co.*, No. 02-CV-0674-L, 2003 U.S. Dist. LEXIS 8455, at *4-5 (S.D. Cal. May 6, 2003) ("[t]he party who resists discovery has the burden to show that discovery should not be allowed, and has the burden of clarifying, explaining, and supporting its objections") (internal citations omitted)). Instead, it must specifically detail why each request is irrelevant, *Schaap v. Executive Indus., Inc.*, 130 F.R.D. 384, 387 (N.D. Ill. 1990), or must make a specific showing of privilege. *Windsor v. Martindale*, 175 F.2d 665, 668 (D. Colo. 1997). The objecting party must also show how each discovery request is burdensome or oppressive by presenting affidavits or other evidence establishing the nature of the burden. *Oleson v. K-Mart Corp,* 175 F.R.D. 560, 565 (D. Kan. 1987) (citing cases).

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Scantibodies has not even tried to meet this burden of showing that Abbott's discovery requests are unduly burdensome, irrelevant or privileged, let alone come close to succeeding in that endeavor. It has utterly failed to meet its heavy burden of proof, and it has no basis to further delay full and complete compliance with Abbott's subpoena.

First and foremost, there is no reasonable dispute that each of the categories of documents and requested deposition testimony sought by Abbott are relevant to one or more of the claims or defenses at issue. Many of the categories of documents requested seek information related to the structure, components and operation of the accused products (*E.g.*, Exh. 2, at Schedule B, Topics 5-18.) Remaining categories are directed to evidence relevant to Abbott's claim of damages against Church & Dwight and/or its claim that Church & Dwight's infringement has been willful. (*E.g.*, *id.* Topics 1-4.) The deposition topics Abbott included in the subpoena are similarly related to those claims, with Topics 1-6 relating to the structure, operation, design and manufacture of the accused products and/or their component parts, and Topics 7-14 related to Abbott's claim for damages or that Church & Dwight's infringement has been willful. (*Id.* at internal Exhibit B.)

Scantibodies has offered only one response about the relevance of these topics: boilerplate objections to relevance in its formal objections that do not attempt to substantively explain why any particular category is too broad. (Exh. 3., at 3-11.) That type of generalized objection cannot justify Scantibodies' non-compliance with its obligations under the Federal Rules of Civil Procedure. *See FDIC v. Garner*, 126 F.3d 1138, 1146 (9th Cir. 1997) (affirming decision to enforce subpoenas because of appellants' failure to specify how the subpoenas were overbroad or unduly burdensome); *see also Thomas v. Hickman*, 1:06-cv-00215-AWI, 2007 U.S. Dist. LEXIS 95796, at *17-19 (E.D. Cal. Dec. 6, 2007) (overruling non-party objections that subpoena was overbroad and sought irrelevant documents because objections were not sufficiently specific as required by the Federal Rules).

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Second, Scantibodies has similarly failed to provide a single valid reason for claiming that Abbott's discovery requests are overbroad. It included unhelpful, boilerplate objections about undue burden in its formal objections, again without supporting the objections with even an ounce of substance. *Id.*

Scantibodies also later argued that document request No. 11 was unduly burdensome because it contains no time limitation. (Exh. 6.) But apart from providing no reasoning as to why it would be unduly burdensome for Scantibodies to provide responsive documents from the entire time it has used the antibodies contained in the accused products, Scantibodies' lone attempt at explaining why the time period should be limited to the last two years makes no sense. It argues in essence that because the antibodies of interest allegedly have a "two-year shelf life," documents concerning testing or evaluation of particular antibodies become null, void and irrelevant at the end of that two years when the antibody itself ceases to be active. (*Id.*) That is simply not the case. As Abbott tried to explain to Scantibodies prior to filing this motion, "[a] document that characterizes an antibody prior to the expiration of its 'shelf-life' does not become irrelevant or of less importance at any time thereafter simply because the antibody itself may no longer be active." (Exh. 9.) Scantibodies' argument to the contrary is like arguing that a witness's sworn deposition testimony must be disregarded because the witness herself is no longer available to repeat her previous testimony.

Scantibodies' only other stated position with respect to burden relates to the allegation that much of the discovery sought by Abbott can be obtained by Church & Dwight. While Abbott does not seek second copies of documents it has received from the defendant in the underlying action action, it is critical to note that Scantibodies has never attempted to identify which documents it has that have already been produced by Church & Dwight – a task that Abbott, of course, cannot itself undertake without first obtaining discovery from Scantibodies. Moreover, Scantibodies' willingness to produce even a small category of documents in response to document request No. 11 indicates that there are responsive documents in Scantibodies' possession that have not previously been produced by

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Church & Dwight. Scantibodies' objection to duplicative discovery therefore cannot provide a justifiable basis for its blatant non-compliance.

IV. CONCLUSION

For the foregoing reasons, Abbott respectfully requests that this Court enter an Order compelling Scantibodies' compliance with Abbott's subpoena by compelling Scantibodies to produce all non-privileged documents responsive to Abbott's subpoena by August 28, 2008, produce a witness for deposition no later than September 12, 2008, and permit Abbott to inspect Scantibodies' facilities on the day immediately preceding the scheduled deposition.

Respectfully submitted,

Dated: August 19, 2008

WINSTON & STRAWN LLP

By: _____

Stephen R. Smerek
Attorneys for Plaintiff
ABBOTT LABORATORIES
E-mail: ssmerek@winston.com

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

DECLARATION OF KEVIN E. WARNER IN SUPPORT OF MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES

I, Kevin E. Warner, declare:

1. I am an attorney at law in the State of Illinois associated with Winston & Strawn LLP, counsel for Plaintiff Abbott Laboratories. I have personal knowledge of the facts set forth herein.

2. The underlying action to which this Motion to Compel relates is a patent case involving two patents owned by SurModics, Inc. and exclusively licensed to Plaintiff Abbott (the "Illinois Action"). (Exh. 1.) The case is pending before the Honorable Matthew F. Kennelly, United States District Judge for the United States District Court for the Northern District of Illinois.

3. The patents-in-suit, U.S. Patent Nos. 5,654,162 and 6,020,147 (Exh. 1 at internal Exhibits A and B) cover various types of immunoassays used to detect the presence of substances in sample fluids. One common embodiment of this technology is home-use pregnancy test kits and tests for the prediction of ovulation that are found, for instance, in local pharmacies.

4. In the case of pregnancy test kits, immunoassays are often designed to detect the presence of a hormone called "hCG" in female urine samples, the presence of which indicates that the woman is pregnant. The tests generally operate through the use of proteins called "antibodies" that naturally bind to hCG, and then detecting labels or tags – such as small colored particles – that are attached to the antibodies. The creation and detection of a visible, colored signal at a test area on the device indicates that the antibodies have bound with hCG, that hCG is present in the sample, and that the test user is pregnant. The absence of any color development means the labeled-antibody did not detect hCG, therefore indicating that the user is not pregnant. Tests to predict when a woman is ovulating operate on the same principle, except that the antibodies in those products detect a hormone indicative of ovulation called leuteinzing hormone, or "LH."

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5. Church & Dwight Co., Inc., the defendant in the underlying case, markets several tests for detecting pregnancy and ovulation under labels such as First Response and Answer. Abbott and SurModics allege that those products infringe certain claims of the '162 and '147 patents.

6. Discovery in the Illinois Action has revealed that Church & Dwight is not itself the manufacturer of the accused products. Many of the accused products are manufactured in California by Scantibodies Laboratory, Inc.

7. Scantibodies, as the manufacturer of the accused products, has important documents related to Abbott's and SurModics' allegations of infringement that Abbott cannot obtain from Church & Dwight. For instance, Scantibodies has documents related to the types and properties of the antibodies used in the accused products. The characterization and properties of those antibodies is relevant to Abbott's and SurModics' infringement claims. As another example, Scantibodies also has manufacturing specifications related to the assembly of the accused devices. Abbott believes Scantibodies also has agreements with Church & Dwight and communications with Church & Dwight that bear on several issues in the case, from Church & Dwight's infringement of the patents-in-suit to the damages owed to Abbott and SurModics.

8. In light of the magnitude of Scantibodies' involvement in the manufacture of the accused products marketed by Church & Dwight, Abbott served on Scantibodies, on April 25, 2008, a subpoena requesting the production of various categories of documents and the designation of one or more witnesses to testify at a deposition on behalf of Scantibodies pursuant to Federal Rule 30(b)(6). (Attached hereto as Exh. 2.) Schedule B of the Subpoena contains the categories of documents and things Abbott seeks from Scantibodies. All categories are directly related to the diagnostic products manufactured by Scantibodies for Church & Dwight that are at issue in this litigation, or to Scantibodies' relationship with Church & Dwight.

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9. Scantibodies first responded to the subpoena by submitting a formal set of boilerplate objections, but not agreeing to produce *any* documents. (Exh. 3.) Instead, Scantibodies hid behind the claim that it should not be "put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to" Abbott's requests "that [Defendant Church & Dwight] might have in its possession, custody or control." (*E.g.*, *id.* at 3.) Scantibodies provided no argument in its objections as to why any of Abbott's document requests or deposition topics was irrelevant to the underlying litigation or, at the very least, not calculated to lead to the discovery of admissible evidence. (*Id.*)

10. Following receipt of Scantibodies' objections on May 20, 2008, and in the interests of avoiding conflict between the parties, the undersigned, counsel for Abbott in the Illinois Action, contacted counsel for Scantibodies by phone in June 2008 in an effort to discuss the nature of Abbott's requests for documents and deposition, and to provide further insight into the types of documents Abbott believed that only Scantibodies, and not Church & Dwight, would have in its possession. Abbott's counsel identified documents concerning the testing of the antibodies in the accused products, samples of the antibodies used in the accused products, and certain communications about Church & Dwight and the accused products, as general categories of documents that were likely only in the possession of Scantibodies. Abbott's counsel indicated that it would welcome production of those documents, and then consider whether Abbott would be in a position to forego further document discovery from Scantibodies. At no time during that conversation or at any time thereafter did counsel for Abbott indicate that it was withdrawing any requests for documents contained in the subpoena or withdrawing its request for a deposition of Scantibodies on the topics listed in the subpoena.

11. Abbott was hopeful that it would soon receive a positive response from Scantibodies, but none came. Abbott called Scantibodies again in June 2008, but after receiving no response to the voice mail it left for Scantibodies' counsel seeking both the

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production of documents and the designation of witnesses, counsel for Abbott wrote to Scantibodies' counsel on June 23, 2008, again requesting both the production of responsive documents and the designation of a witness prior. (Exh. 4.) Scantibodies ignored both that voice mail and the e-mail of June 23 for nearly a month.

12. Abbott again wrote to Scantibodies on July 21, 2008, this time requesting a "meet and confer" should Scantibodies' response be less than full compliance with the subpoena. (Exh. 5.)

13. Scantibodies delayed providing any material responsive to Abbott's subpoena for nearly three months after receiving the subpoena. And when it finally did respond, it was with more of the same delay tactics. Scantibodies responded on July 24, 2008, that it would produce documents responsive to ***one*** of the ***eighteen*** categories of documents contained in Abbott's subpoena, and only if Abbott would provide what amounts to an unreasonable time limitation on that request. Moreover, Scantibodies did not mention the possibility of a deposition, mentioning neither who Scantibodies' Rule 30(b)(6) designee(s) would be nor providing a date for deposition. (Exh. 6.)

14. Abbott made several attempts to call Scantibodies' counsel following that e-mail to address Scantibodies' objection regarding limiting any document production to specified time periods. As Abbott then explained on August 4, 2008, no time limit could be placed on documents relevant to any testing or evaluation of the antibodies used in the accused products. (Exh. 7.) Abbott further explained:

> Documents concerning the antibodies used in any accused C&D Test Kit are relevant, regardless of the date of their creation. For instance, documents concerning the properties and characteristics of the antibodies used in the accused Test Kits are relevant to the extent they were created during the period of SLI's relationship with Church & Dwight. Such documents that relate to the same antibodies and/or cell lines are not irrelevant simply because they may pre-date that relationship.

(*Id.*)

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15. Abbott also corrected Scantibodies' suggestion that producing documents responsive to a single category concerning testing and evaluation documents would suffice to meet Scantibodies' obligations under the subpoena. Abbott made clear that it was "not…withdrawing any or all of its other document requests. To the extent [Scantibodies] has documents responsive to those categories that have not been produced in this litigation by Church & Dwight, we request that you produce those documents as well." (*Id.*) Abbott thus requested full compliance with both the documents and deposition requests contained in the subpoena. (*Id.*)

16. Scantibodies again responded with more delay betraying a lack of good faith. It maintained its decision to produce documents responsive to only ***one of eighteen*** categories in the subpoena, and agreed only to produce such documents dating back an arbitrary two years based on the alleged two year "shelf-life" of the antibodies that may have been analyzed by Scantibodies. (Exh. 8.)

17. The "shelf-life" argument bears no relationship to the relevance of a particular document, however. As Abbott stated in response, "A document that characterizes an antibody prior to the expiration of its 'shelf-life' does not become irrelevant or of less importance at any time thereafter simply because the antibody itself may no longer be active." (Exh. 9.) Abbott also again explained that documents responsive to only one request would not satisfy Scantibodies' obligations pursuant to Federal Rule 45.

18. Scantibodies has never moved this Court or any other to quash the subpoena or issue a protective order.

19. Abbott has also served a second subpoena on Scantibodies including a notice of inspection of Scantibodies' facilities. (Exh. 10.) Scantibodies informed Abbott on August 18, 2008, that it would not permit the requested inspection.

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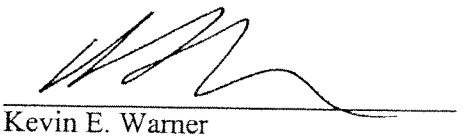
I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

August 19, 2008



Kevin E. Warner

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

EXHIBITS
TO ABBOTT LABORATORIES' MEMORANDUM OF POINTS AND AUTHORITIES IN SUPPORT OF MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES LABORATORY, INC., PURSUANT TO RULE 45(C)(2)(B) AND TO THE DECLARATION OF KEVIN E. WARNER IN SUPPORT THEREOF

TABLE OF CONTENTS

IN THE UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF ILLINOIS

ABBOTT LABORATORIES,

Plaintiff

and

SURMODICS, INC.,

Involuntary Plaintiff

and

INVERNESS MEDICAL SWITZERLAND GMBH,

Involuntary Plaintiff

v.

CHURCH & DWIGHT, INC.

Defendant.

x

07CV3428
JUDGE KENNELLY
MAG.JUDGE NOLAN

COMPLAINT AND JURY DEMAND

Plaintiff ABBOTT LABORATORIES ("Abbott") by and through its undersigned counsel, allege as follows for its Complaint against Defendant Church & Dwight Co., Inc.:

PARTIES

1. Plaintiff Abbott is an Illinois corporation with its principal place of business in Abbott Park, Illinois.

2. Plaintiff SurModics is a Minnesota corporation with its principal place of business in Eden Prairie, Minnesota. Prior to commencing this action, Abbott requested that SurModics join this litigation, but SurModics has not done so. Abbott therefore joins SurModics as an involuntary plaintiff pursuant to Federal Rule of Civil Procedure 19(a).

3. Plaintiff Inverness Medical Switzerland GmbH ("Inverness") is a Switzerland

corporation with, upon information and belief, its principal place of business in the United States in Waltham, Massachusetts. Prior to commencing this action, Abbott requested that Inverness join this litigation, but Inverness has not done so. Abbott therefore joins Inverness as an involuntary plaintiff pursuant to Federal Rule of Civil Procedure 19(a).

4. Upon information and belief, Defendant Church & Dwight Co., Inc. is a Delaware corporation with its principal place of business in New Jersey.

JURISDICTION AND VENUE

5. Jurisdiction for this action is based on 28 U.S.C. §§ 1331 and 1338(a).

6. Venue is proper under 28 U.S.C. §§ 1391(b), (c) and (d) and 1400(b).

7. This Court has personal jurisdiction over Church & Dwight because, among other things, C&D does business in this judicial district and infringing acts that are the subject of this Complaint has occurred in this jurisdiction.

8. This Court has personal jurisdiction over Inverness because it transacts business in this judicial district and the contract, which made Abbott the exclusive licensee of the patents at issue in this litigation, was made with an Illinois corporation and was performed in Illinois.

9. This Court has personal jurisdiction over SurModics because the contract which made Abbott the exclusive licensee of the patents at issue in this litigation, was made with an Illinois corporation and was performed in Illinois

BACKGROUND

10. On August 5, 1997, the PTO duly and legally issued U.S. Patent 5,654,162, (the '162 patent), which is entitled "Chemical analysis apparatus and method." SurModics is the owner of the '162 patent. A true and correct copy of the '162 patent is attached hereto as Exhibit A.

11. The assignee and owner of the '162 patent is Surmodics, Inc., who Abbott has joined as an involuntary plaintiff.

12. Abbott is the exclusive licensee of the '162 patent.

13. Accordingly, Abbott and SurModics have sufficient right, title and interest in the '162 patent to sue for infringement.

14. On February 1, 2000, the PTO duly and legally issued U.S. Patent 6,020,147, (the '147 patent), which is entitled "Chemical analysis apparatus and method." SurModics is the owner of the '147 patent. A true and correct copy of the '147 patent is attached hereto as Exhibit B.

15. The assignee and owner of the '147 patent is Surmodics, Inc., who Abbott has joined as an involuntary plaintiff.

16. Like the '162 patent, Abbott is the exclusive licensee of the '147 patent.

17. Accordingly, Abbott and SurModics have sufficient right, title and interest in the '147 patent to sue for infringement.

18. On March 18, 2003, the PTO duly and legally issued U.S. Patent 6,534,320 (the '320 patent), which is entitled "Process For Immunochromatography With Colloidal Particles." Abbott is the exclusive licensee as it relates to Church & Dwight of the '320 patent. A true and correct copy of the '320 patent is attached hereto as Exhibit C.

19. The '320 patent was originally issued to Abbott, as assignee of the inventors, by the PTO.

20. Abbott was the patent owner until September 30, 2003 when it sold the patents at issue to Inverness Medical Innovations, Inc.

21. Simultaneous with the sale of the patents on September 30, 2003, Inverness

granted Abbott an exclusive license to enforce and sublicense the '320 patent to three "Permitted Sublicensees."

22. Church & Dwight is one of the three "Permitted Sublicensees."

23. The license agreement is in writing.

24. Abbott and Inverness have sufficient right, title and interest in the '320 patent to maintain a suit for infringement of those patents against Church & Dwight.

25. Church and Dwight manufacturers and sells several products that utilize lateral flow technology to detect hormone levels to indicate, *inter alia*, ovulation and pregnancy. (collectively referred to as the "Church & Dwight Products").

COUNT ONE – INFRINGEMENT

'162 Patent

26. Abbott re-alleges and incorporates herein the allegations of paragraphs 1 through 25.

27. Church & Dwight has and still is infringing either directly, contributorily or by inducement, one or more claims of the '162 patent by making, using, selling, offering for sale, and/or otherwise placing the Church & Dwight Products.

28. Church & Dwight's infringement will continue unless enjoined by this Court from further infringement.

29. Abbott has suffered damages as a result of Church & Dwight's infringement of the '162 patent and will suffer additional damage and irreparable harm unless Church & Dwight is enjoined by this Court from continuing/initiating such infringing acts in the future.

30. Upon information and belief, Church & Dwight's acts of infringement have been willful, with full knowledge of the '162 patent and full knowledge that the Church & Dwight Products infringe that patent.

COUNT TWO - INFRINGEMENT

'147 Patent

31. Abbott re-alleges and incorporates herein the allegations of paragraphs 1 through 30.

32. Church & Dwight has and still is infringing either directly, contributorily or by inducement, one or more claims of the '147 patent by making, using, selling, offering for sale, and/or otherwise placing the Church & Dwight Products.

33. Church & Dwight's infringement will continue unless enjoined by this Court from further infringement.

34. Abbott has suffered damages as a result of Church & Dwight's infringement of the '147 patent and will suffer additional damage and irreparable harm unless Church & Dwight is enjoined by this Court from continuing/initiating such infringing acts in the future.

35. Upon information and belief, Church & Dwight's acts of infringement have been willful, with full knowledge of the '147 patent and full knowledge that the Church & Dwight Products infringe that patent.

COUNT THREE - INFRINGEMENT

'320 Patent

36. Abbott re-alleges and incorporates herein the allegations of paragraphs 1 through 35.

37. Church & Dwight has and still is infringing either directly, contributorily or by

inducement, one or more claims of the '320 patent by making, using, selling, offering for sale, and/or otherwise placing the Church & Dwight Products.

38. Church & Dwight's infringement will continue unless enjoined by this Court from further infringement.

39. Abbott has suffered damages as a result of Church & Dwight's infringement of the '320 patent and will suffer additional damage and irreparable harm unless Church & Dwight is enjoined by this Court from continuing/initiating such infringing acts in the future.

40. Upon information and belief, Church & Dwight acts of infringement have been willful, with full knowledge of the '320 patent and full knowledge that the Church & Dwight Products infringe that patent.

RELIEF REQUESTED

WHEREFORE, Plaintiff Abbott Laboratories prays that the Court enter judgment against Defendant Church & Dwight and in favor of Abbott Laboratories as follows:

A. For a finding that Church & Dwight's product infringes one or more claims of the '162, '147, and '320 patents;

B. That the Court enter an injunction barring Defendant, its officers, agents, attorneys, servants employees, and persons acting in active concert or participation with them and who have actual notice of the Order, from selling, offering to sell, placing, distributing, manufacturing, making, importing, exporting, advertising, using, contributing to the use or inducing the use of products that fall within one or more claims of the '162, '147, and '320 patents;

C. That the Court declare this an exceptional case and award Plaintiff its reasonable costs, expenses, and attorneys' fees pursuant to 35 U.S.C. § 285; and

D. For such other relief as the Court determines to be just and proper.

JURY DEMAND

Plaintiff Abbott Laboratories requests a jury trial on all issues so triable.

WINSTON & STRAWN LLP
Attorneys for Defendant,
Abbott Laboratories

By: _____

COUNSEL

George C. Lombardi
Raymond Perkins
Stephanie McCallum
Kevin Warner
WINSTON & STRAWN LLP
35 West Wacker Drive
Chicago, IL 60601

EXHIBIT A

US005654162A

United States Patent [19]

Guire et al.

[11] Patent Number: 5,654,162

[45] Date of Patent: *Aug. 5, 1997

[54] **CHEMICAL ANALYSIS APPARATUS AND METHOD**

[75] Inventors: **Patrick E. Guire**, Eden Prairie; **Melvin J. Swanson**, Carver, both of Minn.

[73] Assignee: **Bio-Metric Systems, Inc.**, Eden Prairie, Minn.

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,073,484.

[21] Appl. No.: **891,932**

[22] Filed: **Jun. 1, 1992**

Related U.S. Application Data

[60] Continuation of Ser. No. 609,794, Nov. 6, 1990, which is a division of Ser. No. 467,229, Feb. 23, 1983, Pat. No. 5,073,484, which is a continuation-in-part of Ser. No. 356,459, Mar. 9, 1982, abandoned.

[51] **Int. Cl.**[6] **G01N 33/543**; G01N 33/558

[52] **U.S. Cl.** **435/7.92**; 435/7.94; 435/7.95; 435/970; 436/514; 436/518; 436/810

[58] **Field of Search** 435/7.92–7.95, 435/970, 805; 436/514, 518, 169, 810; 422/56

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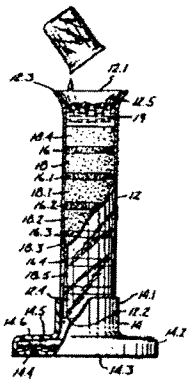
Primary Examiner—David Saunders

Attorney, Agent, or Firm—Fredrikson & Byron, P.A.

[57] **ABSTRACT**

A method and apparatus for the determination of an analyte in a liquid sample, the method and apparatus employing a liquid-permeable solid medium defining a liquid flow path. The medium includes one or more reactant-containing reaction zones spaced apart along the flow path and in which reaction occurs with the analyte or an analyte-derivative (e.g. a labelled analyte) to result in the formation of a predetermined product. Detector means are employed to detect analyte, analyte derivative, reactant or predetermined product in one or more reaction zones, the number of such zones in which such detection occurs reflecting the presence of analyte in the liquid.

30 Claims, 2 Drawing Sheets



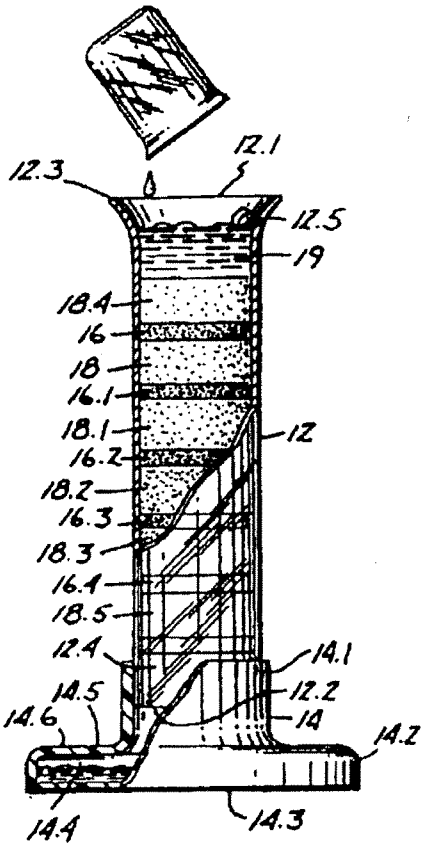


Fig. 1

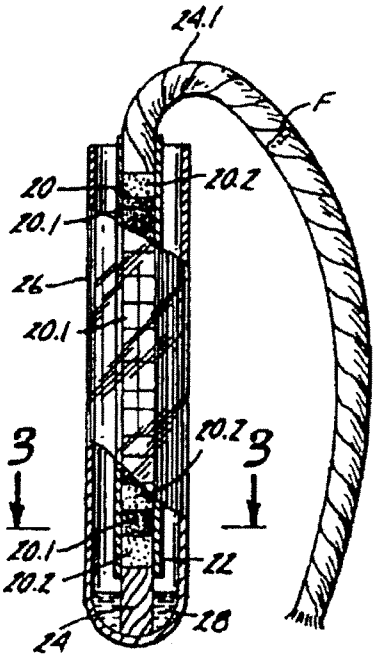


Fig. 2

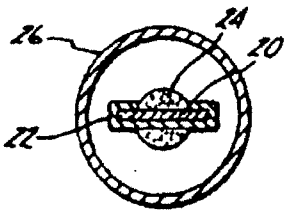
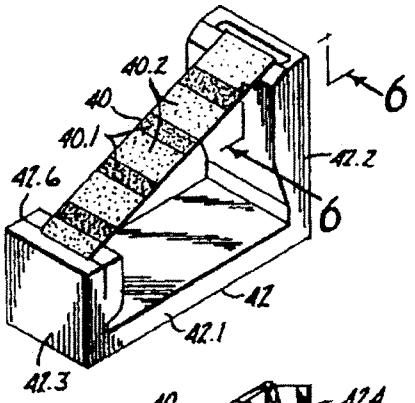


Fig. 3

Fig.5



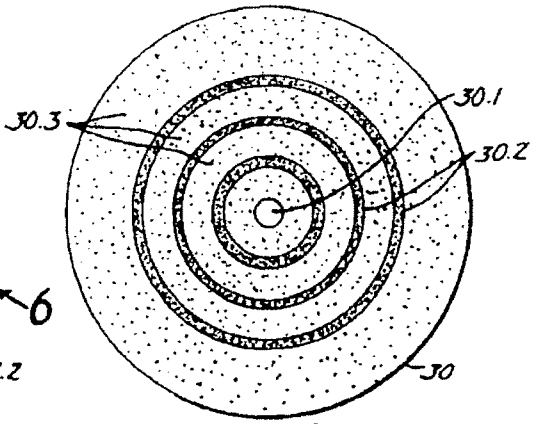


Fig.4

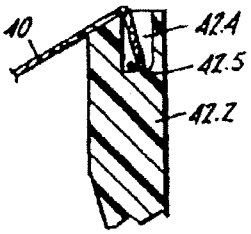


Fig.6

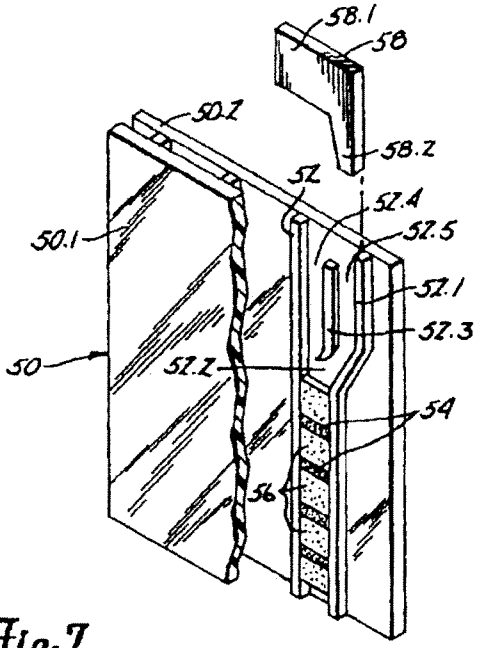


Fig.7

CHEMICAL ANALYSIS APPARATUS AND METHOD

This is a continuation of Ser. No. 609,794, filed Nov. 6, 1990, which is a divisional of Ser. No. 467,229, filed Feb. 23, 1983, now U.S. Pat. No. 5,073,484, which is a continuation in part of Ser. No. 356,459, filed Mar. 9, 1982, abandoned.

FIELD OF THE INVENTION

The invention is in the field of quantitative chemical analysis, and is particularly useful in the detection and analysis of small amounts of chemical substance in such biological fluids as milk, blood, urine, etc.

DESCRIPTION OF THE PRIOR ART

Procedures for quantitatively determining the concentration of chemical substances in solutions are legion. Many of these procedures are long and tedious, and are highly susceptible to human error. Many procedures involve the reaction of the chemical moiety—the analyte—to be detected with a reactant to form a product, the procedures including a step of determining the amount of reactant that is consumed (e.g., as in titrations), or the amount of product that is produced (e.g., as by measuring the absorption of light by the product of a chromogenic reaction), or as by measuring the amount of the chemical moiety or reaction product that can be separated from the solution (e.g., by distillation), etc. Some quantitative analysis procedures, such as are used in radioimmunoassays, involve competitive reactions between labeled analytes (e.g., labeled with radioisotopes of iodine, enzymes, or fluorescent, chromogenic or fluorogenic molecules) in known quantities and unknown amounts of unlabeled analytes, the amount of analyte in the unknown solution being related to the measured radioactivity or other property of a specimen resulting from the test after suitably separating the reacted or bound analyte from the unreacted or unbound analyte, or through properties of the bound and unbound labeled analyte that permit them to be distinguished. Many of such procedures involve changes in color (as when chemical indicators are employed that respond by color changes to differences in hydrogen ion concentration), or in turbidity (as when the procedure involves the formation of a solid reaction product).

Certain analyses involve the passage of a fluid, such as air, through a column containing a reactant which may change color upon contact with an ingredient of the air. For example, U.S. Pat. No. 3,286,506 describes a gas analyzing technique in which a measured amount of gas is passed through a glass cartridge containing an indicator, the amount of gas to be detected being proportional to the amount of indicator within the column that changes color. Similar devices are shown in U.S. Pat. Nos. 3,312,527 and 3,545,930.

There is a recurring trend in the field to provide analytical procedures which are characterized by speed, simplicity, and by a reduction in the vulnerability of such procedures to human error. Simple, rapid tests, for example, have been marketed for determining the approximate level of blood sugar for diabetics. Such tests, however, often are relatively imprecise. It would be highly desirable to provide a quantitative test for chemical moieties that on the one hand would be characterized by high sensitivity and that yet on the other hand would be characterized by simplicity, rapidity and relative freedom from human error.

SUMMARY OF THE INVENTION

In one embodiment, the invention provides an apparatus for the quantitative analysis of a chemically reactive substance (hereafter referred to as an "analyte"), in a carrier fluid such as a liquid. The apparatus includes a fluid-permeable solid medium that has a predetermined number of successive, spaced reaction zones and which defines a path for fluid flow sequentially through such zones. "Fluid" herein is typified as a liquid. Predetermined quantities of a reactant are bound to the solid medium in such zones and are capable of reaction with the analyte or with an analyte derivative, to result in the formation of a predetermined product. The apparatus may further include detector means for detecting, in the spaced zones, the presence Of the analyte or its derivative, the reactant, or the predetermined product resulting from the reaction between the analyte or its derivative and the reactant. In addition, the apparatus may include means for suppressing the detectability of trace amounts of the analyte or its derivative, the reactant, or the predetermined product resulting from the reaction between the analyte or its derivative and the reactant.

As used herein, the terms "reactant", "reactive" and the like when used in connection with the reaction between the analyte or its derivative and the reactant refers to the ability of the reactant to react, by covalent or hydrogen bonding or by any other means, with the analyte or its derivative to form or result in the formation of a predetermined product. That is, such terms are used in their broadest sense as referring to the ability of the reactant to in any way act upon, be acted upon, or interact with the analyte or analyte derivative in a manner that detectably alters the analyte or its derivative, the reactant or both to thereby result in the formation of a reaction product. Similarly, "reaction product" means any product resulting from the reaction of the analyte or its derivative and the reactant and that is detectably different from both. "Analyte derivative" means a chemical moiety derived from an analyte, and desirably is a tagged or labeled form of the analyte as may be employed in analytical procedures involving competing reactions between an analyte and its tagged or labeled derivative.

In the apparatus of the invention, the reactant is bound to the permeable solid medium in the successive, spaced zones through which the analyte passes. A procedure employing the apparatus may take the form in which the analyte or its derivative, as it passes through the reaction zones, becomes bound to the reactant and the presence of the analyte or its derivative within the reaction zones is detected, as by color change or the like. Similarly, in a slightly modified embodiment, the analyte or its derivative may react with the reactant to result in the formation of a product that itself remains bound in the reaction zones, and the product itself is then detected. In these embodiments, one may determine with considerable precision the concentration of the analyte by detecting how many of the successive reaction zones, beginning with the upstream zone, show the presence of the analyte or its derivative, or of the product resulting from the reaction between the reactant and the analyte or analyte derivative. In another embodiment, the reactant that is bound to the permeable solid medium may itself be capable of detection by suitable detection means and may be disabled from such detection when reacted with an analyte or analyte derivative. In this manner, as the analyte or analyte-analyte derivative composition passes through successive reaction zones, the reactant in the successive zones is disabled from such detection until substantially all of the analyte or analyte-analyte derivative composition has been exhausted, while remaining downstream reaction zones still contain reactant that can be detected. In a modified form, the reaction between the analyte or analyte derivative and the reactant may cause the latter to become unbound from the

solid medium to which it was attached and hence be washed from the successive zones. When the analyte or analyte derivative or both has thus been exhausted, subsequent or downstream reaction will display reactant that is yet bound to the permeable medium and which can be detected. In such embodiments, one may count the number of zones in which the reactant has been disabled beginning with the upstream zone.

As used herein, "analyte" refers not only to the particular chemical moiety for which analysis is desired, but also to chemical moieties that are reaction products of the moiety to be determined with another chemical moiety. For example, a biological fluid containing an unknown amount of a chemical moiety may be reacted in solution or otherwise with another chemical moiety to provide a product, the concentration of which is related to the initial concentration of the chemical moiety to be measured. The resulting product, then, may become the "analyte" for use in the apparatus and method of the invention. Accordingly, "analyte" refers to any chemical moiety which is to be measured quantitatively.

In a preferred embodiment, the invention employs immunochemical reactions in which the analyte and the reactant represent different parts of a specific ligand-antibody (antiligand) binding pair. Members of such a specific binding pair may be referred to herein as a "sbp members".

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a broken-away view, in partial cross-section, showing an apparatus of the invention;

FIG. 2 is a broken-away view, in partial cross-section, showing another apparatus of the invention;

FIG. 3 is a cross-sectional view taken along line 3—3 of FIG. 2;

FIG. 4 is a plan view of another embodiment of an apparatus of the invention;

FIG. 5 is a perspective view of yet another test apparatus of the invention;

FIG. 6 is a broken-away cross sectional view taken along line 6—6 of FIG. 5; and

FIG. 7 is a perspective, broken-away view of another test apparatus of the invention.

DETAILED DESCRIPTION

With reference to FIG. 1, a transparent, hollow column of glass or the like is designated 12 and has open top and bottom ends (12.1, 12.2). The top end (12.1) preferably is flared outwardly as shown at (12.3). A standard (14) is provided at the bottom end of the column, and may have a hollow, upstanding portion (14.1) into which the bottom portion (12.4) of the column may be snugly fitted as by a press fit. The standard includes a relatively wide bottom portion (14.2) having a flat, horizontal surface such as a table. The interior (14.4) of the standard preferably is hollow, and an upper wall (14.5) of the standard preferably is provided with a breathing aperture (14.6) so as to permit air to escape from the column when liquid is poured into the upper end (12.1) of the column. The aperture (14.6) may, if desired, be fitted with a loose, porous plug, such as a cotton plug, to retard leakage from the device when it has been disposed of in a trash container or the like. It may also, if desired, be fitted with a flexible tubing which may connect it with a pump (e.g., peristaltic, syringe drive withdrawal, etc.) typifying flow control means to control the flow rate of the liquid through the assay column.

Within the column are positioned successive, spaced reaction zones (16, 16.1, 16.2, 16.3, etc.), occupied by a permeable solid medium such as beaded agarose, beaded polyacrylamide, porous glass, cellulose or other materials permeable to liquid and compatible with the analyte, analyte derivative, reactant and detector means. To the medium in the reaction zones is bound a reactant, as will be described more fully below. The interior of the column, as will now be understood, describes a generally vertical liquid flow path, and the permeable solid medium positioned in the reaction zones desirably occupies the entire cross-section of the flow path. Between the spaced reaction zones are positioned preferably nonreactive spacer layers (18, 18.1, 18.2, 18.3 and so on) of a liquid-permeable solid medium through which liquid may flow, the spacer layers preferably being in intimate contact with the reaction zones. The spacer layers desirably are of the same permeable solid medium as the reaction zones, and, preferably, spacer layers 18.4, 18.5 are provided at the top and bottom of the columns as well so that each reaction zone is sandwiched between spacer layers. At its upper end, the column (12) may be provided with an aperture (12.5) spaced a given distance above the spacer layer (18.4) so as to provide a predetermined volume between the aperture and top surface of the spacer layer. In a known manner, as a liquid (represented as (19) in the drawing) is poured into the open upper end (12.1) of the column, it will occupy the open volume at the top of the column and any amount of the liquid (20) in excess of that desired may escape outwardly through the aperture (12.5), thereby insuring that no more than a given, predetermined amount of the liquid passes downwardly in the column. The space (20) may, if desired, be filled or partially filled with a porous, nonreactive material such as glass wool or similar material to avoid splashing of the liquid within the upper end of the column.

To the liquid-permeable solid medium within the spaced reaction zones (16, 16.1, etc.) is bound a reactant that is reactive with a given analyte or analyte derivative to form a product, all in accordance with the above definitions and as exemplified herein. As a typical example, the reactant and analyte may be so chosen that the analyte or its derivative becomes chemically attached to the reactant as the solution (20) of analyte or analyte and analyte derivative solution (the "test solution") passes downwardly through the column, care being taken that the total amount of reactant within the column is in excess of that required to so react with the expected quantity of analyte and analyte derivative in the solution. After the test solution has begun its passage downwardly through the column, a wash solution, typically distilled and deionized water, optionally may be poured into the open end of the column to further aid the downward passage of the test solution through the column. Finally, an indicator or detector material that detects the presence of analyte or analyte derivative, reaction product or reactant, as by causing a color change within the zones (16, 16.1 and so on) may be poured into the upper end of the column. As the test solution flows downwardly through the column, predetermined amounts of the analyte or its derivative are reacted with or bound to the reactant in each such layer until the analyte or its derivative have been exhausted from the solution. The concentration of analyte in the solution can be determined by merely counting the number of successive zones, beginning at the top of the column, that have changed color. In another emobidment, the reactant that is bound to the medium in the zones (16, 16.1 and so on) may be deactivated or disenabled by reaction with the analyte or analyte derivative or both, and the detector which is

employed may determine, as by a color change, the presence of nondisabled reactant. In this embodiment, the reactant in successive zones contacted by the test solution will be disabled until analyte and analyte derivative have been exhausted from the solution. Upon detecting which of the reaction zones contain reactant that has not been disabled, one may determine the concentration of analyte in the solution by counting the number of zones beginning at the top of the column in which reactant is not detected. Of course, in this embodiment as in the embodiment set out above, one may also count the number of zones beginning with the bottom of the column as well.

Another physical embodiment of the apparatus of the invention is shown in FIGS. **2** and **3** in which "wicking", or upward capillary flow of a liquid through a strip of filter paper or similar material is employed, the strip having successive, spaced reaction zones. In this embodiment, the permeable solid medium may take the form of a strip of filter paper, which is designated generally as (**20**) in FIGS. **2** and **3**. Within spaced zones (**20.1**) of the filter paper strip is bound a reactant, as above-described, the spaced zones being separated by spacer layers or sections (**20.2**). One method of preparing the strip (**20**) involves binding a reactant to small, individual rectangular paper filter pieces, and then alternating these pieces, which form the reaction zones, with similar pieces of filter paper that do not contain the reactant, the alternating pieces of filter paper being held together, for example, by a thin strip of adhesive tape. Other, more sophisticated methods of manufacture will be apparent to those skilled in the art.

As shown in FIGS. **2** and **3**, the strip (**20**) of filter paper may be positioned in an elongated plastic holder (**22**) having a generally C-shaped cross-section. The bottom of the holder is adapted to receive the end of a wick (**24**) which may consist of twisted strands of cotton or other fibrous material. A similar wick (**24.1**) is received in the upper end of the plastic holder. The ends of the wicks (**24**, **24.1**) contact the ends of the filter strip (**20**). As shown in FIG. **2**, the upper and lower sections of the filter paper strip which come into contact with the wicks (**24**, **24.1**), are spacer layers (**20.2**) so that each reaction zone (**20.1**) is sandwiched between spacer layers (**20.2**). The filter paper strip and holder are adapted for insertion into a test tube (**26**) or other container so that the lower wick (**24**) contacts the bottom of the test tube and the upper wick (**24.1**) extends out of the test tube and then downwardly toward its bottom, all as shown in FIG. **2**. A test solution (**28**) placed in the bottom of the test tube (**26**) is thus caused to flow by capillary action upwardly through the length of the filter paper strip, successively contacting the reaction zones (**20.1**) in a manner analogous to the flow of test solution through the column depicted in FIG. **1**. As will be described more fully below, the filter paper strip and holder can be moved from one test tube to another so that different solutions can be caused to sequentially flow through its length.

Referring now to FIG. **4**, an apparatus of the invention is embodied in a disc of permeable, solid medium such as filter paper, porous glass, etc. (**30**). The disc (**30**) may be placed horizontally in a suitable container such as a petri dish. At its center, the disc (**30**) is provided with a well (**30.1**) to receive a test solution or other solution. Reaction zones, spaced radially from the well (**30.1**), are shown as annular rings (**30.2**), and are separated from one another by spacer layers also in the form of annular rings (**30.3**). Spacer layers preferably form the innermost and outermost rings of the disc. The reaction zones (**30.2**) and the spacer layers (**30.3**) are concentric. Test solution that is admitted to the central well (**30.1**) is thus carried radially outwardly of the well by capillary action or by diffusion, aided by centrifugal force if desired, the test solution successively passing through the spaced reaction zones (**30.2**).

FIGS. **5** and **6** show another embodiment of a device of the invention. The device includes a filter paper strip (**40**) similar to that of FIGS. **2** and **3** and containing spaced reaction zones (**40.1**) separated by spacer layers (**40.2**). A holder, preferably of plastic, is designated (**42**) and has a flat base (**42.1**) with upwardly extending legs (**42.2**, **42.3**) carried at its ends. The leg (**42.2**) is provided with an upwardly open well (**42.4**) into which may be inserted the upper end of the filter paper strip (**40**), care being taken that the end (**42.5**) of the filter paper extends downwardly to the floor of the well. The strip of filter paper extends obliquely downwardly from the well, and its lower end is captured in a slot (**42.6**) formed in the leg (**42.3**). In use, the test solution or other solution is placed in the well (**42.4**), and is carried downwardly of the strip by both gravity and capillary action, the solution sequentially encountering the spaced reaction zones (**40.1**).

FIG. **7** shows yet another embodiment of a device of the invention which can be used for multiple concurrent tests. The device, designated (**50**), includes a pair of spaced plates (**50.1**, **50.2**). Referring to the right-hand portion of FIG. **7**, the space between the plates is divided into generally vertical channels by means of elongated spacers (**52**, **52.1**). As shown in the drawing, the channel (**52.2**) formed by the spacers has a wide upper section and a narrow lower section. The lower section is provided with a series of vertically spaced reaction zones (**54**) comprising a liquid-permeable solid medium to which is bound a reactant, the medium being any of those described above. Between the reaction zones, are placed spacer layers (**56**), the spacer layers sandwiching between them the reaction zones (**54**). Between the spacers (**52**, **52.1**), at the upper end of the channel is placed an elongated vertical divider (**52.3**) which divides the upper portion of the channel into two sections (**52.4**) and (**52.5**). A plug (**58**), which may be made of the same material as the spacers, has an upper, finger-gripping portion (**58.1**) and a lower, tapering plug portion (**58.2**) adapted to be inserted in the channel (**52.5**). The flat surfaces of each of the spacers and plug, of course, contact the facing surfaces of both glass plates to prevent leakage of material from the channels.

In use, a solution such as a test solution is poured into the upper end of the channels formed by the spacers (**52**, **52.1**), and the plug (**58**) is then inserted to provide an air-tight upper seal in the one channel (**52.5**). As a result, liquid in the other channel (**52.4**) preferentially flows downwardly through the reaction zones and spacer layers. When the liquid level in the channel (**52.4**) falls below the lower end of the spacer (**52.3**), air can bubble upwardly through the channel (**52.5**), permitting the contents of that channel to empty downwardly through the reaction zones as well. In this manner, the sequential flow of liquid, first from channel (**52.4**) and then from channel (**52.5**), is rendered automatic. Preferably, one of the plates (e.g., plate **50.1**) is transparent so that the results of any color change in the reaction zones may readily be observed. The other plate (**50.2**) may be transparent or may be of an opaque white or other light color to serve as a background against which color changes can readily be seen.

ANALYTES-REACTANTS

Analytes that can be detected in accordance with the present invention include substantially all chemical sub-

stances that are reactive with a reactant to form a product, as above discussed. It will be understood that the invention is not limited to any particular analyte or reactant, but is useful for substantially any analyte-reactant combination.

Many analytes may be analyzed simply by adapting known chemical reactions to the invention.

For example, carbon dioxide may be analyzed with phenolphthalein at a slightly alkaline pH. Calcium ion may be analyzed utilizing a reactant comprising calmodulin and mammalian phosphodiesterase or another calmodulin-sensitive enzyme (Maekawa and Abe, *Biochemical and Biophysical Research Communications* 97:621 (1980)). Ferrous ion may be analyzed utilizing, as a reactant, a ferrocene derivative (Katz, et al. J. Am. Chem. Soc. 104:346 (1982)). A large number of additional examples may be selected from the list of organic analytical reagents compiled by John H. Yoe in *Handbook of Chemistry and Physics*, p.D 126–129. 57th Edition, Robert C. Weast, Ed., CRC Press, Cleveland. 1976. and in other references cited therein.

Typical analyte-reactant pairs selected from the field of organic chemistry similarly may be chosen by adapting known chemical reactions to the invention. For example, almost any phenol may be analyzed with Gibbs Reagent (2.6-dichloro-p-benzoquinone-4-chlorimine) (Dacre, J., Analytical Chemistry 43:589 (1971)). A reagent for Indoles is p-dimethylaminobenzaldehyde (Fieser and Fieser, *Reagents For Organic Synthesis*, Volume 1, p.273, John Wiley & Sons, Inc., New York. (1967). The last-mentioned reference also shows the use of phenylhydrazine as a reactant for cortisone and similar steroids, and the use of sulfoacetic acid as a reactant in the Liebermann-Burchard test for unsaturated sterols. Amino acids and ammonium salts may be analyzed using the reagent ninhydrin (indane-1.2.3-trione hydrate) (Pasto, et al *Organic Structure Determination*, p.429. Prentice-Hall, Inc., Englewood Cliffs, N.J., 1969). Reducing sugar may be measured with Red Tetrazolium (2.3.5-triphenyl-2H-tetrazolium Chloride) (Fieser. Organic Experiments, p.135, Raytheon Education Co., Lexington, Mass., 1968).

Various other analyte reagent pairs may be selected from the field of chemistry for adaptation to the invention from such reference works as: Schuurs, et al, U.S. Pat. No. 3,654,090 (Enzyme-Linked Immunosorbent Assay); Kay, U.S. Pat. No. 3,789,116 (Fluorescent Labeled Antibody Reagents); Rubenstein, et al, U.S. Pat. No. 3,817,837 (Homogeneous Enzyme Immunoassay); Ling. U.S. Pat. No. 3,867,517 (Radioimmunoassay); Glaever, U.S. Pat. No. 3,906,490 (Radial Immunodiffusion); Ullman, U.S. Pat. No. 3,996,345 (Flourescence Quenching Homogenous Immunoassay); Maggio, U.S. Pat. No. 4,233,402 (Enzyme Channeling Homogeneous Enzyme Immunoassay); Boguslaski, et al, Canadian Patent 1,082,577 (Hapten-Cofactor Homogeneous Enzyme Immunoassay); Schonfeld, H., Ed., "New Developments in Immunoassays". *Antibiotics and Chemotherapy*, Volume 26, 1979; O'Sullivan, et al, "Enzyme Immunoassays: A Review", *Annals of Clinical Biochemistry* 16:221 (1979); Schuurs, et al, *Enzyme Immunoassay*, Clin. Chim. Acta. 81:1 (1977); Feldmann, et al, Eds., *First International Symposium On Immunoenzymatic Techniques*, INSERM Symp. No. 2, North Holland Publishing Co., Amsterdam, 1976; Williams, et al, *Methods in Immunology and Immunochemistry*, Volume 3, Academic Press, New York. 1971; and Yalow, et al, J. Clin. Invest. 39:1157 (1960).

Yet other analyte-reactant pairs may be found in: reference works such as Feigl, F., *Spot Tests in Inorganic Analysis*, 6th edition. Elsevier Publishing Co., New York, 1972; Feigl. Fritz, *Spot Tests in Organic Analysis*, 7th edition, Elsevier Publishing Co., New York, 1966; Snell, F. and Snell, C., *Colorimetric Methods of Analysis*, Vols. 1–4AAA, Van Nostrand Reinhold Co., New York. 1967–74; and Braibanti, A., Ed. *Bioenergetics and Thermodynamics: Model Systems—Synthetic and Natural Chelates and Macrocycles as Models for Biological and Pharmaceutical Studies*, D. Reidel Publishing Co., Boston. 1980.

The references identified above are incorporated herein by reference.

Of particular importance to the instant invention are those analyte-reactant combinations that form specific binding pairs of which one is an antibody and in which the other is a ligand to which the antibody is specific.

Such Immunochemical reactant pairs are well-known in the art, and a wide variety of tests have been devised to detect the presence or quantity or both of an analyte, particularly when the analyte is present only in exceedingly small concentrations. Reference is made to the above-identified patents and publications.

DETECTORS

The detectors useful in the invention are capable of detecting the presence in the successive reaction zones, of analyte, analyte derivatives, reactants, or the predetermined reaction product, all as described above. The means of detection may take various forms. In the preferred embodiment, detection is signaled by a change of color, or a lack of a change of color, in the respective reaction zones of the apparatus. However, detection may be signaled by other means as well, such as by luminescence or fluorescence of the zones, radioactivity of the zones, etc. For many reactions, detection is signaled by a change in pH, and the detector may hence take the form of a pH color indicator such as phenolphthalein, Nile Blue A, Thymol Blue, and Methyl Violet. In other tests, one may detect the presence or absence of the appropriate chemical moiety in a reaction zone by observing whether a solid reaction product has settled upon the succeeding spacer layer. Various detector mechanisms are known to the art, and need not be described in detail. In the preferred embodiment, however, which makes use of immunochemical reaction between the analyte or the analyte and its derivative and the reactant, often very small concentrations of analyte are to be measured and accordingly a magnifying or amplifying mechanism may suitably be employed. One such mechanism makes use of enzymes to promote the reaction of a reaction product with a detector moiety to in turn provide a visual color indication. For example, the analyte to be tested may be provided in admixture with a known quantity of an analyte-glucose oxidase conjugate as an analyte derivative, the spaced, sequential reaction zones of the apparatus containing an antibody specific to the analyte. A signal generating system, such as horseradish peroxidase bound to the antibody in the permeable solid medium in such zones and a chromogenic material such as o-dianisidine (added. with glucose, to the test solution) can be employed. The addition of the test solution, containing the analyte, the analyte-glucose oxidase conjugate, glucose, catalase and o-dianisidine, is then flowed through the apparatus, such as the column depicted in FIG. 1. The analyte and the analyte-glucose oxidase conjugate compete for binding sites on the bound antibody, thereby resulting in a color formation due to the reaction of the o-dianisidine with hydrogen peroxide produced by the glucose oxidase—catalyzed reaction of oxygen with glu-

cose. Unreacted analyte and analyte-glucose oxidase conjugate flow to successive zones until the admixture has been exhausted of analyte and analyte-glucose oxidase conjugate. A variety of modifications of this procedure, of course, are known to the art.

EXAMPLE I

The chromogen 5,5'[3-(2-Pyridyl)-1.2.4-triazine-5,6-diyl] bis-2-furansulfonic acid, disodium salt, ("Ferene", a trademarked product of Chemical Dynamics Corp.) is used for the determination of serum iron in soluble assays through measurement of the absorbance at 593 nm, at which wavelength any interference from other pigments in the serum should be minimized. It may be covalently coupled to useful carrier derivatives through nitration, reduction, diazotization and diazonium coupling to proteins (such as albumin) immobilized on agarose beads, paper strips or other suitable permeable solid medium. This immobilized signal-generating reagent (chromogenic chelating agent) is physically arranged in sequential spaced layers or bands (reaction zones) through which the test fluid will migrate.

Small columns are prepared from silanized Pasteur pipettes by breaking off both ends, attaching a short piece of tubing to the bottom (constricted) end and inserting glass wool plugs in the tube bottoms. The columns are packed by sequential insertion of layers of agarose-Ferene separated by layers of unmodified agarose. Typically, 0.4 ml of a 1:1 agarose suspension is applied directly above the support, followed by alternating layers of 50 microliters of a 1:1 suspension of agarose-Ferene and 0.2 ml of agarose suspension. After each addition to the column, the walls of the columns are rinsed with phosphate buffered saline (PBS) and the solution above the gel is allowed to flow into the gel before addition of the next layer.

For use in the assay, the tubing at the bottom of the prepared column is connected to a peristaltic pump to control the flow rate of the assay. An appropriate dilution of test sample for iron analysis is applied to the column. The iron solution ("test" solution) is passed through the assay column at controlled flow rates, typically between 10 and 15 minutes for complete entrance. When all the solution is into the gel bed, the columns are rinsed with water. As the test solution flows through, color develops in some of the Ferene-containing reaction zones. The number of colored zones resulting is a function of the concentration of iron in the test solution.

EXAMPLE II

A. The enzyme cholinesterase reacts with and is inhibited by toxic organophosphate and carbamate agents. Cholinesterase and the chromogenic sulfhydryl reagent 5.5'-Dithiobis-(2-nitrobenzoic acid) (Ellman's Reagent) are immobilized upon agarose beads, which are then assembled into columns according to Example I. A test solution (diluted blood serum) is added to the column and migrates through the reaction zones following which a solution of butyrylthiocholine iodide is added. The zones retaining catalytically active cholinesterase will generate a yellow color through reaction of the thiocholine produced by the hydrolytic activity of the cholinesterase, with the immobilized Ellman's Reagent. The presence of reactive cholinesterase-inhibiting toxin in the test sample will result in fewer colored bands, which will be in the downstream end region of the column.

B. Amino acids and other nucleophilic amine compounds are measured by use of the chromogenic reagent 2.4-dinitrofluorobenzene (FDNB), which produces a yellow product upon reaction. A 0.1 ml aqueous sample, containing about 0.1–1.0 micromoles of amino analyte, is transferred to a siliconed glass vessel. The pH is adjusted to 7.0, if necessary, and two milligrams (25 micromoles) of $NaHCO_3$ is added and dissolved. Next is added 0.12 ml of 0.15% FDNB in absolute alcohol (1.5 micromoles). This solution is prepared fresh shortly before use. After the reaction has neared completion, it is analyzed for remaining FDNB (reactant) content by flow exposure to the assay system prepared according to Example I. In this case, a similar amine-containing analyte is immobilized in the zones in a permeable solid medium at a known content (e.g., 0.1–0.25 micromoles per reaction zone). After rinsing with 50% ethanol in aqueous solution, the number of yellow reaction zones produced and remaining after rinse will be inversely related to the amount of analyte in the test sample.

EXAMPLE III

The IgG fraction from rabbit anti-penicilloylbovine gamma globulin was partially purified by precipitation with 33% saturated ammonium sulfate. The precipitate was redissolved and dialyzed against phosphate buffered saline (PBS). This IgG preparation was used for immobilizing antibodies onto beaded agarose. The agarose was suspended in dioxane, then reacted with carbonyldiimidazole. After being washed with dioxane, it was suspended in water, and then in aqueous borate buffer, pH 9.0. The IgG was then added to the activated agarose and the gel suspension stirred by rocking at 4° C. for 2 days. After extensive washing with PBS, the gel containing immobilized antibody was ready for use in the assay.

Small columns were prepared from silanized Pasteur pipettes by breaking off both ends, attaching short pieces of tubing to the bottom (constricted) ends and inserting glass wool plugs in the column bottoms. The columns were packed by sequentially inserting alternating layers of agarose-IgG separated by layers of unmodified agarose. Typically 0.4 ml of 1:1 agarose suspension was applied directly above the plugs, followed by alternating layers of 50 microliters of a 1:1 suspension of agarose-IgG (to form the reaction zones) and 0.2 ml of agarose suspension (to form spacer layers). After each addition, the walls of the columns were rinsed with PBS and the solution above the gel was allowed to flow into the gel before addition of the next layer.

For use in an assay, tubing at the bottom of the prepared column was connected to a peristaltic pump to control the flow rate of the assay. An appropriate dilution of penicilloyl-glucose oxidase ("Pen-GO") (typically 0.1 microgram Pen-GO in 1 ml PBS) with or without known amounts of the analyte (penicilloyl-epsilon amino caproate) (Pen-EAC), was applied to the column. The Pen-GO was prepared by reacting penicillin G with glucose oxidase in borate buffer, pH 9.0, for 2–3 days at 4° C. The Pen-GO solution was passed through the assay column at controlled flow rates, typically between 10 and 15 minutes for complete entrance into the gel. When all the solution was into the gel bed, a detector solution was added to the column. The detector solution was prepared as follows: 0.20 ml of horseradish peroxidase (HRP) solution (2 mg/ml), 2 ml of 18% glucose solution, 1 ml of 0.2M phosphate buffer (pH 6.0) and 0.100 ml of 1% o-dianisidine was diluted 1:10 in PBS and 1 ml or less was applied to the columns at the same flow rate as the previous solutions. Brown color developed in some of the reaction zones. Presence of the penicilloyl moiety in the Pen-GO solution results in the upper reactive zone or zones being lighter in color, with color being generated in zones further down the column.

This Example may be repeated for the analysis of serum albumin (a large protein molecule) by replacement of the penicillin-glucose oxidase conjugate with an albumin-glucose oxidase conjugate.

EXAMPLE IIIA

Peroxidase-labeled IgG prepared from rabbit antiserum against penicillin was immobilized in small strips of filter paper by the method reported in Example III. Catalase was bound to other, similar strips of filter paper. The first and second mentioned strips were then cut into rectangular shapes to provide, respectively, reaction zones and spacer layers. The small rectangular pieces of filter paper were then layed onto a strip of adhesive tape, alternating the reaction zones and spacer layers with edges of the sequential pieces of paper overlapping or at least touching one another to provide a continuous capillary flow path.

Penicilloyl-glucose oxidase ("Pen-GO") in a solution of Human Serum Albumin ("HSA") was freeze-dried inside a test tube. Within another test tube, made of brown glass for protecting the contents from light, was freeze-dried a solution of o-dianisidine and glucose in phosphate-buffered saline at pH 6.0.

A short wick was attached to the bottom of the prepared filter paper strip described above, and a longer wick was placed in contact with the upper end of the strip. The strips themselves can be stored under refrigeration, and preferably are retained in a wet condition resulting from the preparation described above.

In one example of use, a test solution consisting of a measured volume of milk containing a known concentration of penicillin G is added to the test tube containing the freeze-dried Pen-GO, and the tube is shaken gently to mix the contents. The filter paper strip is then inserted into the tube with its upper, longer wick extending over the lip of the tube and then downwardly as shown in FIG. 2. When the entire solution has been taken up by the strip alternatively, when the solution reaches an arbitrary flow line marked on the upper wick and designated "F" in FIG. 2) the filter paper strip is removed from the test tube and is placed in the brown glass tube to which previously has been added water to dissolve the freeze-dried contents thereof. The latter solution similarly wicks upwardly through the filter paper strip, causing color development to occur in certain of the reaction zones as determined by the quantity of penicillin G in the initial test solution.

In this example, the penicillin G in the milk and the penicillin of the Pen-GO compete for binding sites on the antibodies immobilized in the reaction zones of the filter paper strip. Of course, larger concentrations of penicillin G in the milk sample cause the penicillin G and the Pen-GO to migrate further through the filter paper strip. The presence of Pen-GO in any of the zones is indicated through the development of color from the reaction of H_2O_2 with o-dianisidine, the H_2O_2 being formed from the glucose oxidase in the presence of glucose and oxygen, and as catalyzed by the peroxidase. The catalase in the spacer layers catalyzes the conversion of H_2O_2 into O_2 and H_2O, and thus prevents migration of H_2O_2 from one reactive zone to another.

As with each of the apparatuses described herein, the device of this example may be calibrated by determining how many of the reaction zones become colored as a result of the test procedure. For example, one of the reaction zones may change color only when the test solution (e.g., milk) contains at least 9 nanograms of analyte (e.g., penicillin G) per ml. For a sample of milk containing an unknown concentration of penicillin G, one merely counts the number of reaction zones that have changed color to find the narrow, defined concentration range within which lies the penicillin G concentration.

EXAMPLE IV

Antibody against a polyvalent antigen (e.g., serum albumin) analyte is labeled with peroxidase and bound to permeable solid medium according to Example III to form reaction zones in a column. Another batch of the same or similar antibody is labeled with an enzyme such as glucose oxidase. Into the column is poured a test sample containing an unknown amount of analyte antigen. Through the column is then flowed the soluble glucose oxidase-antibody in the presence of glucose plus catalase plus o-dianisidine. The number of colored bands resulting is directly related to the amount of analyte antigen in the test sample relative to the antigen binding capacity of the antibody zones. In this example, the antigen first reacts with the bound antibody and binds to the antibody, forming a predetermined product. The latter, in turn, is detected by the coupling of the glucose oxidase antibody conjugate to available antigenic sites on the antigen followed by the color forming reaction.

EXAMPLE V

An analyte or a derivative thereof (e.g., penicillin-peroxidase) is covalently bound to a permeable solid medium according to Example III. An enzyme-labeled receptor (e.g., glucose oxidase-antibody against penicillin) is prepared and exposed to the immobilized analyte to form the specific binding complex (e.g., immune complex). The assay unit is assembled according to Example III. Subsequent exposure to a test sample containing an unknown amount of analyte is done at elevated temperature (e.g., 60° C.) to hasten the attainment of equilibrium through competitive binding of the immobilized analyte and analyte in the test sample with the enzyme-labeled antibody. Analyte in the test sample under such conditions will competitively displace the labeled antibody from the immobilized analyte. The number of colored reaction zones resulting from the procedure is inversely related to the amount of analyte in the test sample. These bands will appear in the terminal or downstream portion of the column.

EXAMPLE VI

Three assay columns with 4 reaction zones each were prepared according to Example III, except that the top reaction zone was prepared with 75 microliters of IgG-agarose suspension (1:1) and the lower 3 zones with 50 microliters. Test samples containing 0, 50 and 200 nanograms Pen-EAC, were placed in different columns, with each test sample containing 200 ng Pen-GO per ml. Flow time for sample application was 20 min. Application of the solution of signal generating reagents produced 2 colored zones with the 0 ng Pen-EAC sample, 3 in the 50 and 4 with the 200 ng sample.

A wider and more precise range of analyte content, of course, may be measured by using a larger number of assay zones.

In a preferred embodiment, only a single pass through the apparatus of a single liquid material is required. An analyte may be mixed with an analyte derivative, chromogen or other material and flowed through the apparatus to yield an appropriate test result. In a further preferred embodiment, the apparatus is chemically complete in that it includes all

reactants and other chemicals necessary or desirable for the quantitative analysis of an analyte; that is, all that is required is that the analyte in a liquid carrier be flowed through the apparatus. Elements of the apparatus that, if combined, would undergo reaction in the absence of the analyte may be maintained in different zones. For example, the bottom-most layer (20.2) of the strip of FIG. 2 may contain a reactant physically separated from reactants in the adjacent reaction zone. When the analyte in a carrier liquid is flowed through the layer (20.2), the reactant in this layer together with the analyte and carrier liquid is flowed into the first reaction zone. If desired, a reactant may be provided in the form of a solid and may merely be placed upon the upper layer (18.4) of the column of FIG. 1, the reactant being dissolved by and carried with the liquid carrier and analyte into the column.

The above-described embodiments are typified by the following Examples VII–IX which also describe and exemplify a preferred format of the invention.

This format requires at least two enzymes, one of which is coupled to an analyte to form an analyte derivative and catalyzes a color-forming reaction, and another enzyme that is immobilized in reaction zones which also contain antibody to the analyte, the latter enzyme providing substrate for the color-generating enzyme. In this format, therefore, only a single solution which consists of or contains the analyte test sample is flowed into or through the solid medium after which color develops in the reaction zones, the number of colored zones being directly related to the concentration of analyte in the test sample.

EXAMPLE VII

The IgG fraction from rabbit anti-penicilloylbovine serum albumin was partially purified by precipitation with 33% saturated ammonium sulfate. This protein was coupled to microcrystalline cellulose by reaction of the cellulose with carbonyldiimidazole in dioxane, followed by washing and then by reaction with the IgG preparation in borate buffer at pH 9.0 at 4 degrees C. for two days. The cellulose was then washed extensively with PBS and used for preparation of banded strips. Glucose oxidase was also coupled to microcrystalline cellulose in the same manner. A penicilloyl-peroxidase was prepared by first coupling a polyacrylamide amine to HRP, then reacting penicillin G with that preparation. It is believed that the use of a linear polymer as a spacer for attaching the hapten to the enzyme allows more hapten molecules to be coupled to each enzyme molecule and renders the hapten molecules more accessible for binding to antibody, thus speeding the binding rate. Polyacrylamide was synthesized by dissolving 0.5 gm. of acrylamide in 200 ml. of deionized water, degassing, then adding 0.2 ml. of N,N,N',N'-tetramethylethylenediamine and 0.15 gm. of ammonium persulfate. This solution was mixed, then allowed to sit at room temperature for 30 min. then passed through an ultrafiltration membrane, dialyzed vs. deionized water and lyophilized. The polyacrylamide was then dissolved in 1.0 ml. of 0.2M phosphate buffer at pH 7.7 and 0.3 ml. of 25% glutaraldehyde was added. This solution was incubated at 37 degrees C. for 19 hours after which it was passed through a Sephadex G-25 column to remove the excess glutaraldehyde. The void volume fractions which absorbed strongly at 230 nm were pooled and added to a solution of diaminodipropylamine (0.5 ml. in 2.0 ml. of water) at pH 9.0. This solution was allowed to react at 4 degrees C. over night. The reaction mixture was then passed through a Sephadex G-150 column and the fractions that absorbed significantly at 230 nm. were divided into four pools of equal volume, the second of which was coupled to peroxidase (HRP). HRP was reacted with 1.25% glutaraldehyde at pH 7.0 for 15 hours at room temperature. After passing the reaction mixture through a Sephadex G-25 column, the HRP-containing fractions were pooled and added to the polyacrylamide-diamine preparation, the pH was adjusted to 9.0, and this solution was allowed to react at 4 degrees C. overnight. The peroxidase-polyacrylamide-diamine was then passed through a Biogel P-100 column and the void volume fractions were pooled and concentrated, then reacted with penicillin. Fifty mg. of penicillin G was added to the peroxidase-polyacrylamide-diamine, the pH adjusted to 9.0 and stirred at 4 degrees C. over night. This preparation was then dialyzed extensively, then used for the assay.

Banded strips were prepared by cutting 0.5×8.0 cm. strips of a polyester film having a hydrophilic surface onto which were glued strips of Whatman 3MM chromatography paper. At one end was glued a 8.5×4.0 cm. long paper strip followed by a 3.5 mm. space. Then three one cm. long paper strips were glued onto the Mylar strip with 2.0 mm. spaces between them. The paper on the Mylar was wetted with a solution of 0.02% o-dianisidine in water. The spaces were then filled in with a suspension of microcrystalline cellulose prepared by mixing 50% suspensions of the IgG-cellulose and the glucose oxidase-cellulose in a 20:1 ratio. The first space was filled with 20 ul. of this suspension and the other three spaces each contained 10 ul. These strips were air dried, then stored dry until used.

The strips were developed by placing the end with the longer paper spacer into a small vial containing the developing solution. This solution contained peroxidase-polyacrylamide-diamine-penicillin (25 ul. of a 0.25 microgram/ml. solution), glucose (0.3 ml of a 1.125% glucose solution in 0.2M phosphate buffer at pH 6.0) and 10 ul. of dilutions of penicilloyl-aminocaproic acid (EAC) in water. Under these conditions, pink bands could readily be observed after 20–30 min., such that, with no penicilloyl-EAC in the developing solution, one band was colored; with 0.4 micromolar hapten (penicilloyl-EAC), two bands were colored; and with 1.0 uM penicilloyl-EAC, all three bands were colored.

If needed or desired, antibody to peroxidase, an HRP-binding lectin or some other binder or inactivator of peroxidase can be included in the spacer layers for the purpose of improving the sharpness or decisiveness of zone color determinations. Furthermore, catalase immobilized in the spacer layers may permit more rapid color development in the reaction zones without generation of color in the spacer layers.

EXAMPLE VIII

Banded strips are prepared according to Example VII, except that all of the components of the assay except the sample to be tested are incorporated into the strip. The peroxidase-polyacrylamide-diamine-penicillin is dissolved in a solution of between 0.5 and 1.0% gelatin containing 2.5% glucose and 0.2M phosphate buffer at pH 6.0, 0.1 ml. of which is applied to the bottom paper strip and dried. In this example, therefore, the user has only to dip the strip into a solution suspected of containing the analyte, wait for a prescribed time, then read the results by counting the number of colored bands on the strip.

EXAMPLE IX

Assay columns are prepared according to Example III, except that the reaction zones are composed of a mixture of

IgG-agarose and glucose oxidase-agarose (20:1). Peroxidase-penicillin (as prepared in Example VII), glucose, o-dianisidine, and phosphate buffer, stored in dry form, are dissolved in 1.0 ml of the test sample which is then added to the column and allowed to flow through. The results are read after the prescribed time by counting the number of colored bands on the column. The reagents added to the analyte test sample can be in the form of a small pellet or can be dried onto the under surface of the cap for a small vessel used to measure the volume of sample, etc. In the latter case, the vessel is filled, the cap placed on top, the vessel inverted a few times and the sample is poured into the column. The reagents to be mixed with the sample can even be dried onto a small plug that is stored in the top of the column, in which case they dissolve when the sample is added to the column.

Various other enzyme pairs can be used for generating color in the reaction zones. For example, alkaline phosphatase can be immobilized in the reaction zones with beta-galactosidase coupled to the analyte. The use of naphthol-beta-D-galactopyranoside-6-phosphate as substrate for the alkaline phosphatase results in the generation of naphthol-beta-D-galactopyranoside, which is hydroloyzed by beta-galactosidase to produce naphthol which in the presence of a diazonium salt results in a colored product in the reaction zones.

The accuracy and reliability of the apparatus of the invention depends to some extent upon how readily or easily the generation of color or other detectable change in the different reaction zones may be ascertained. A reaction zone in the direction of analyte flow desirably should show detectable changes only when a significant, minimum quantity of analyte or other material being detected has passed through the preceeding reaction zone; since the physical nature of the apparatus often does not permit reaction to go fully to completion in each such zone, a small "tail" e.g., trace, amount of material may flow into successive zones and may be marginally detected in such zones to yield readings that are difficult to interpret. One may largely avoid this problem, however by several means. Detectors may be employed that are sensitive only to minimum concentrations of a chemical moiety to be detected. For example, one may utilize o-phenylene diamine in place of o-dianisidine as a chromophore in the above examples, the former being less sensitive. Another method involves the placement in spacer layers or, less desirably, in reaction zones, of small quantities of "scavenger" reactants capable of immobilizing or deactivating trace amounts of materials, as exemplified in Example VII. This enables the sensitivity and operation of the apparatus to be tailored as desired to particular analyses. Control of sensitivity and reliability also may depend upon the concentration of the reactant in the solid reaction zones, and the solubility of materials such as the colored product in some analyses.

While a preferred embodiment of the present invention has been described, it should be understood that various changes, adaptations and modifications may be made therein without departing from the spirit of the invention and the scope of the appended claims.

What is claimed is:

1. A device generating a signal indicative of the presence of an analyte in a liquid solution suspected of containing said analyte, said device comprising:

(a) a liquid permeable solid medium comprising a solution contact portion and one or more spaced reactive zones separated from said contact portion;

(b) a solution suspected of containing said analyte and having traversed said medium, including said reactive zone(s);

(c) a reactant non-diffusively bound to said medium only at said reactive zone(s), said reactant being specific for and bound to said analyte or a reaction product comprising said analyte and a chemical moiety; and

(d) a labeled antibody specific for and bound to said analyte or said reaction product in said reactive zone(s); wherein said device provides a detectable signal in said reactive zone(s) as an indication of the presence or absence of said analyte in said solution.

2. A device according to claim 1 wherein said labeled antibody is labeled with an enzyme and is detected by adding a substrate for said enzyme.

3. A device according to claim 2 wherein the bound labeled antibody is specific for the analyte.

4. A device according to claim 3 wherein the bound reactant is specific for the analyte.

5. A device according to claim 1 wherein the solution contact portion is a well into which said carrier liquid has been placed.

6. A device according to claim 5 which is adapted such that said carrier liquid has been drawn out of said well by capillary action of said liquid permeable solid medium.

7. A device according to claim 1 wherein the bound labeled antibody is specific for the analyte.

8. A device according to claim 7 wherein the bound reactant is specific for the analyte.

9. A method for detecting the presence of an analyte in a carrier liquid suspected of containing said analyte, which method comprises;

a) providing a liquid permeable solid medium which defines a path for fluid flow capable of supporting capillary flow, along which are i) a site for application of the carrier liquid, ii) a diffusively bound labeled antibody specific for the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety, said antibody being capable of flow along the flow path, and iii) one or more zones spaced along said flow path, each zone having a predetermined amount of a reactant bound to it which is specific for either the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety;

b) contacting said application site with said carrier liquid such that the liquid passes along the flow path by capillary flow such that analyte or reaction product of the analyte with another chemical moiety becomes bound to both the labeled antibody and the reactant bound to the solid medium; and

c) detecting the labeled antibody which, together with the reactant bound to the solid, medium, has sandwiched the analyte or chemical moiety as an indication of the presence of analyte.

10. The method of claim 9 wherein the solid permeable medium contains all the materials needed for detecting the presence of the analyte so that all that is required is the application of the carrier liquid.

11. A method according to claim 9 wherein said labeled antibody is labeled with an enzyme and is detected by adding a substrate for said enzyme.

12. A method according to claim 11 wherein the diffusively bound labeled antibody is specific for the analyte.

13. A method according to claim 12 wherein the bound reactant is specific for the analyte.

14. A method according to claim 9 wherein said site for application of said carrier liquid is a well into which said carrier liquid is placed.

15. A method according to claim 14 wherein said carrier liquid is drawn out of said well by the capillary action of said liquid permeable solid medium.

16. A method according to claim 9 wherein said carrier liquid passes along said flow path as a single material in a single pass.

17. A method according to claim 16 wherein the diffusively bound labeled antibody is specific for the analyte.

18. A method according to claim 17 wherein the bound reactant is specific for the analyte.

19. A method according to claim 9 further comprising a chemical moiety diffusively bound along said flow path, said moiety being reacted with said analyte in order to form a reaction product.

20. A method according to claim 9 wherein the diffusively bound labeled antibody is specific for the analyte.

21. A method according to claim 20 wherein the bound reactant is specific for the analyte.

22. A device for detecting the presence of an analyte in a carrier liquid suspected of containing said analyte, which device comprises a liquid permeable solid medium which defines a path for fluid flow capable of supporting capillary flow, along which are i) a site for application of the carrier liquid, ii) a diffusively bound labeled antibody specific for the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety, said antibody being capable of flow along the flow path, and iii) one or more zones spaced along said flow path, each zone having a predetermined amount of a reactant bound to it which is specific for either the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety;

wherein said device can be used by contacting a carrier liquid with said application site in such a manner that permits said liquid to pass along the flow path by capillary flow such that analyte or reaction product of the analyte with another chemical moiety becomes bound to both the labeled antibody and the reactant bound to the solid medium; and wherein the labeled antibody, with the reactant bound to the solid medium, sandwiches the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety.

23. A device according to claim 22 wherein said labeled antibody is labeled with an enzyme and is detected by adding a substrate for said enzyme.

24. A device according to claim 23 wherein the diffusively bound labeled antibody is specific for the analyte.

25. A device according to claim 24 wherein the bound reactant is specific for the analyte.

26. A device according to claim 22 wherein said site for application of said carrier liquid is a well into which said carrier liquid is placed.

27. A device according to claim 26 which is adapted such that said carrier liquid is drawn out of said well by the capillary action of said liquid permeable solid medium.

28. A device according to claim 22 further comprising a chemical moiety diffusively bound along said flow path, said moiety being capable of reacting with said analyte in order to form a reaction product.

29. A device according to claim 22 wherein the diffusively bound labeled antibody is specific for the analyte.

30. A device according to claim 29 wherein the bound reactant is specific for the analyte.

* * * * *

EXHIBIT B

US006020147A

United States Patent [19]

Guire et al.

[11] Patent Number: 6,020,147

[45] Date of Patent: *Feb. 1, 2000

[54] **CHEMICAL ANALYSIS APPARATUS AND METHOD**

[75] Inventors: **Patrick E. Guire**, Eden Prairie; **Melvin J. Swanson**, Carver, both of Minn.

[73] Assignee: **SurModics, Inc.**, Eden Prairie, Minn.

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **07/891,864**

[22] Filed: **Jun. 1, 1992**

Related U.S. Application Data

[60] Continuation of application No. 07/574,607, Aug. 28, 1990, abandoned, which is a division of application No. 06/467,229, Feb. 23, 1983, Pat. No. 5,073,484, which is a continuation of application No. 06/356,459, Mar. 9, 1982, abandoned.

[51] **Int. Cl.**[7] **G01N 33/543**; G01N 33/558; G01N 33/566

[52] **U.S. Cl.** **435/7.92**; 435/7.94; 435/7.95; 435/970; 436/501; 436/514; 436/518; 436/810

[58] **Field of Search** 435/7.92, 7.94, 435/7.95, 970; 436/514, 518, 810, 501; 422/56

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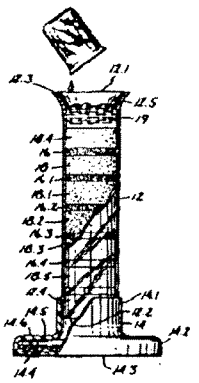
Primary Examiner—David Saunders
Attorney, Agent, or Firm—Fredrikson & Byron, P.A.

[57] **ABSTRACT**

A method and apparatus for the quantitative determination of an analyte in a liquid employs a liquid-permeable solid medium defining a liquid flow path. The medium includes a number of reactant-containing reaction zones spaced apart along the flow path and in which reaction occurs with the analyte or an analyte derivative (e.g., a labeled analyte) to result in the formation of a predetermined product. Detector means are employed to detect analyte, analyte derivative, reactant or predetermined product in the reaction zones, the number of such zones in which such detection occurs indicating the amount of analyte in the liquid.

12 Claims, 2 Drawing Sheets



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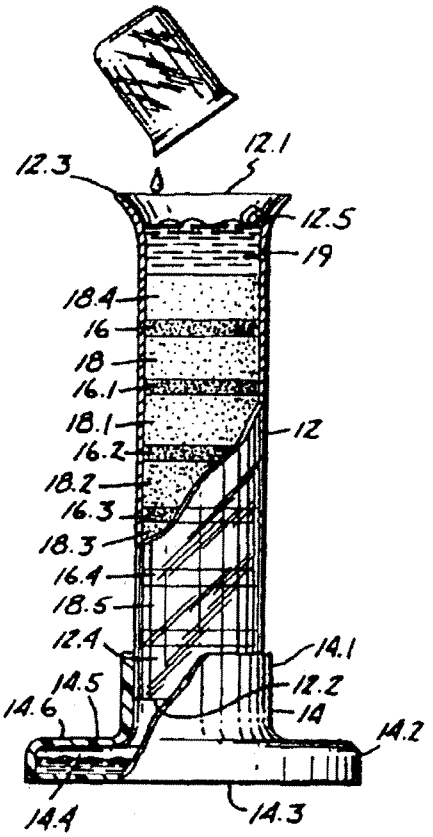


Fig. 1

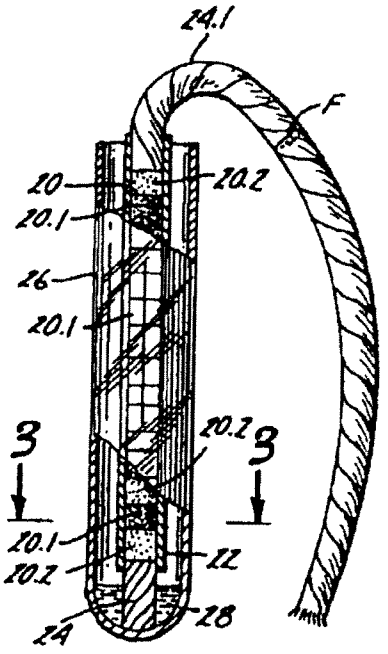


Fig. 2

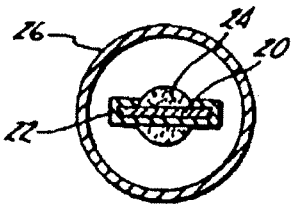
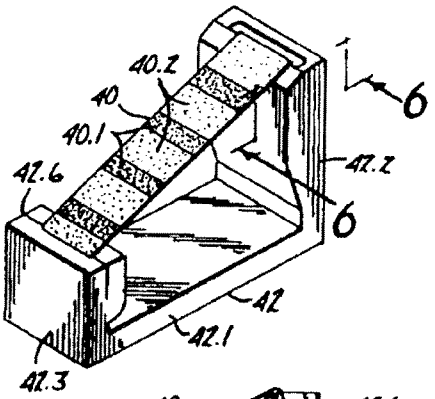


Fig. 3

Fig.5



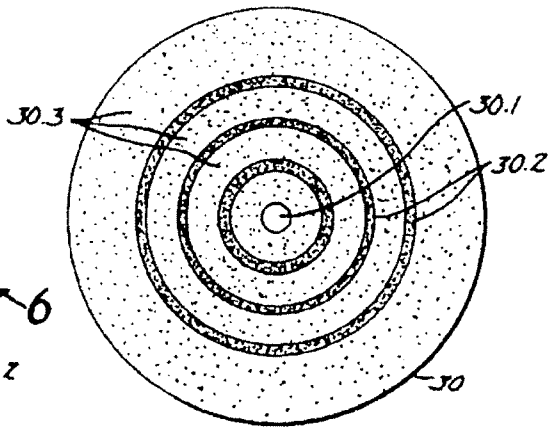


Fig.4

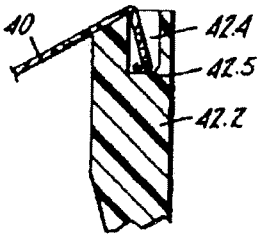


Fig.6

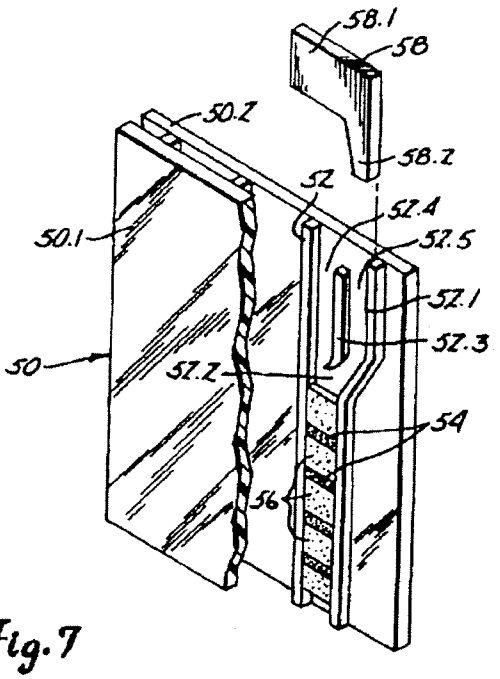


Fig.7

CHEMICAL ANALYSIS APPARATUS AND METHOD

This is a continuation of Ser. No. 574,607 now abandoned, filed Aug. 28, 1990, which is a divisional of Ser. No. 467,229, filed Feb. 23, 1983, now U.S. Pat. No. 5,073,484, which is a continuation in part of Ser. No. 356,459, filed Mar. 9, 1982, abandoned.

FIELD OF THE INVENTION

The invention is in the field of quantitative chemical analysis, and is particularly useful in the detection and analysis of small amounts of chemical substance in such biological fluids as milk, blood, urine, etc.

DESCRIPTION OF THE PRIOR ART

Procedures for quantitatively determining the concentration of chemical substances in solutions are legion. Many of these procedures are long and tedious, and are highly susceptible to human error. Many procedures involve the reaction of the chemical moiety—the analyte—to be detected with a reactant to form a product, the procedures including a step of determining the amount of reactant that is consumed (e.g., as in titrations), or the amount of product that is produced (e.g., as by measuring the absorption of light by the product of a chromogenic reaction), or as by measuring the amount of the chemical moiety or reaction product that can be separated from the solution (e.g., by distillation), etc. Some quantitative analysis procedures, such as are used in radioimmuno-assays, involve competitive reactions between labeled analytes (e.g., labeled with radioisotopes of iodine, enzymes, or fluorescent, chromogenic or fluorogenic molecules) in known quantities and unknown amounts of unlabeled analytes, the amount of analyte in the unknown solution being related to the measured radioactivity or other property of a specimen resulting from the test after suitably separating the reacted or bound analyte from the unreacted or unbound analyte, or through properties of the bound and unbound labeled analyte that permit them to be distinguished. Many of such procedures involve changes in color (as when chemical indicators are employed that respond by color changes to differences in hydrogen ion concentration), or in turbidity (as when the procedure involves the formation of a solid reaction product).

Certain analyses involve the passage of a fluid, such as air, through a column containing a reactant which may change color upon contact with an ingredient of the air. For example, U.S. Pat. No. 3,286,506 describes a gas analyzing technique in which a measured amount of gas is passed through a glass cartridge containing an indicator, the amount of gas to be detected being proportional to the amount of indicator within the column that changes color. Similar devices are shown in U.S. Pat. Nos. 3,312,527 and 3,545,930.

There is a recurring trend in the field to provide analytical procedures which are characterized by speed, simplicity, and by a reduction in the vulnerability of such procedures to human error. Simple, rapid tests, for example, have been marketed for determining the approximate level of blood sugar for diabetics. Such tests, however, often are relatively imprecise. It would be highly desirable to provide a quantitative test for chemical moieties that on the one hand would be characterized by high sensitivity and that yet on the other hand would be characterized by simplicity, rapidity and relative freedom from human error.

SUMMARY OF THE INVENTION

In one embodiment, the invention provides an apparatus for the quantitative analysis of a chemically reactive substance (hereafter referred to as an "analyte"), in a carrier fluid such as a liquid. The apparatus includes a fluid-permeable solid medium that has a predetermined number of successive, spaced reaction zones and which defines a path for fluid flow sequentially through such zones. "Fluid" herein is typified as a liquid. Predetermined quantities of a reactant are bound to the solid medium in such zones and are capable of reaction with the analyte or with an analyte derivative, to result in the formation of a predetermined product. The apparatus may further include detector means for detecting, in the spaced zones, the presence of the analyte or its derivative, the reactant, or the predetermined product resulting from the reaction between the analyte or its derivative and the reactant. In addition, the apparatus may include means for suppressing the detectability of trace amounts of the analyte or its derivative, the reactant, or the predetermined product resulting from the reaction between the analyte or its derivative and the reactant.

As used herein, the terms "reactant", "reactive" and the like when used in connection with the reaction between the analyte or its derivative and the reactant refers to the ability of the reactant to react, by covalent or hydrogen bonding or by any other means, with the analyte or its derivative to form or result in the formation of a predetermined product. That is, such terms are used in their broadest sense as referring to the ability of the reactant to in any way act upon, be acted upon, or interact with the analyte or analyte derivative in a manner that detectably alters the analyte or its derivative, the reactant or both to thereby result in the formation of a reaction product. Similarly, "reaction product" means any product resulting from the reaction of the analyte or its derivative and the reactant and that is detectably different from both. "Analyte derivative" means a chemical moiety derived from an analyte, and desirably is a tagged or labeled form of the analyte as may be employed in analytical procedures involving competing reactions between an analyte and its tagged or labeled derivative.

In the apparatus of the invention, the reactant is bound to the permeable solid medium in the successive, spaced zones through which the analyte passes. A procedure employing the apparatus may take the form in which the analyte or its derivative, as it passes through the reaction zones, becomes bound to the reactant and the presence of the analyte or its derivative within the reaction zones is detected, as by color change or the like. Similarly, in a slightly modified embodiment, the analyte or its derivative may react with the reactant to result in the formation of a product that itself remains bound in the reaction zones, and the product itself is then detected. In these embodiments, one may determine with considerable precision the concentration of the analyte by detecting how many of the successive reaction zones, beginning with the upstream zone, show the presence of the analyte or its derivative, or of the product resulting from the reaction between the reactant and the analyte or analyte derivative. In another embodiment, the reactant that is bound to the permeable solid medium may itself be capable of detection by suitable detection means and may be disabled from such detection when reacted with an analyte or analyte derivative. In this manner, as the analyte or analyte-analyte derivative composition passes through successive reaction zones, the reactant in the successive zones is disabled from such detection until substantially all of the analyte or analyte-analyte derivative composition has been exhausted.

while remaining downstream reaction zones still contain reactant that can be detected. In a modified form, the reaction between the analyte or analyte derivative and the reactant may cause the latter to become unbound from the solid medium to which it was attached and hence be washed from the successive zones. When the analyte or analyte derivative or both has thus been exhausted, subsequent or downstream reaction will display reactant that is yet bound to the permeable medium and which can be detected. In such embodiments, one may count the number of zones in which the reactant has been disabled beginning with the upstream zone.

As used herein, "analyte" refers not only to the particular chemical moiety for which analysis is desired, but also to chemical moieties that are reaction products of the moiety to be determined with another chemical moiety. For example, a biological fluid containing an unknown amount of a chemical moiety may be reacted in solution or otherwise with another chemical moiety to provide a product, the concentration of which is related to the initial concentration of the chemical moiety to be measured. The resulting product, then, may become the "analyte" for use in the apparatus and method of the invention. Accordingly, "analyte" refers to any chemical moiety which is to be measured quantitatively.

In a preferred embodiment, the invention employs immunochemical reactions in which the analyte and the reactant represent different parts of a specific ligand-antibody (antiligand) binding pair.

DESCRIPTION OF THE DRAWINGS

FIG. **1** is a broken-away view, in partial cross-section, showing an apparatus of the invention;

FIG. **2** is a broken-away view, in partial cross-section, showing another apparatus of the invention;

FIG. **3** is a cross-sectional view taken along line 3—3 of FIG. **2**;

FIG. **4** is a plan view of another embodiment of an apparatus of the invention;

FIG. **5** is a perspective view of yet another test apparatus of the invention;

FIG. **6** is a broken-away cross sectional view taken along line 6—6 of FIG. **5**; and

FIG. **7** is a perspective, broken-away view of another test apparatus of the invention.

DETAILED DESCRIPTION

With reference to FIG. **1**, a transparent, hollow column of glass or the like is designated **12** and has open top and bottom ends (**12.1**, **12.2**). The top end (**12.1**) preferably is flared outwardly as shown at (**12.3**). A standard (**14**) is provided at the bottom end of the column, and may have a hollow, upstanding portion (**14.1**) into which the bottom portion (**12.4**) of the column may be snugly fitted as by a press fit. The standard includes a relatively wide bottom portion (**14.2**) having a flat, horizontal surface such as a table. The interior (**14.4**) of the standard preferably is hollow, and an upper wall (**14.5**) of the standard preferably is provided with a breathing aperture (**14.6**) so as to permit air to escape from the column when liquid is poured into the upper end (**12.1**) of the column. The aperture (**14.6**) may, if desired, be fitted with a loose, porous plug, such as a cotton plug, to retard leakage from the device when it has been disposed of in a trash container or the like. It may also, if desired, be fitted with a flexible tubing which may connect it with a pump (e.g., peristaltic, syringe drive withdrawal, etc.) typifying flow control means to control the flow rate of the liquid through the assay column.

Within the column are positioned successive, spaced reaction zones (**16**, **16.1**, **16.2**, **16.3**, etc.), occupied by a permeable solid medium such as beaded agarose, beaded polyacrylamide, porous glass, cellulose or other materials permeable to liquid and compatible with the analyte, analyte derivatives reactant and detector means. To the medium in the reaction zones is bound a reactant, as will be described more fully below. The interior of the column, as will now be understood, describes a generally vertical liquid flow path, and the permeable solid medium positioned in the reaction zones desirably occupies the entire cross-section of the flow path. Between the spaced reaction zones are positioned preferably nonreactive spacer layers (**18**, **18.1**, **18.2**, **18.3** and so on) of a liquid-permeable solid medium through which liquid may flow, the spacer layers preferably being in intimate contact with the reaction zones. The spacer layers desirably are of the same permeable solid medium as the reaction zones, and, preferably, spacer layers **18.4**, **18.5** are provided at the top and bottom of the columns as well so that each reaction zone is sandwiched between spacer layers. At its upper end, the column (**12**) may be provided with an aperture (**12.5**) spaced a given distance above the spacer layer (**18.4**) so as to provide a predetermined volume between the aperture and top surface of the spacer layer. In a known manner, as a liquid (represented as (**19**) in the drawing) is poured into the open upper end (**12.1**) of the column, it will occupy the open volume at the top of the column and any amount of the liquid (**20**) in excess of that desired may escape outwardly through the aperture (**12.5**), thereby insuring that no more than a given, predetermined amount of the liquid passes downwardly in the column. The space (**20**) may, if desired, be filled or partially filled with a porous, nonreactive material such as glass wool or similar material to avoid splashing of the liquid within the upper end of the column.

To the liquid-permeable solid medium within the spaced reaction zones (**16**, **16.1**, etc.) is bound a reactant that is reactive with a given analyte or analyte derivative to form a product, all in accordance with the above definitions and as exemplified herein. As a typical example, the reactant and analyte may be so chosen that the analyte or its derivative becomes chemically attached to the reactant as the solution (**20**) of analyte or analyte and analyte derivative solution (the "test solution") passes downwardly through the column, care being taken that the total amount of reactant within the column is in excess of that required to so react with the expected quantity of analyte and analyte derivative in the solution. After the test solution has begun its passage downwardly through the column, a wash solution, typically distilled and deionized water, optionally may be poured into the open end of the column to further aid the downward passage of the test solution through the column. Finally, an indicator or detector material that detects the presence of analyte or analyte derivative, reaction product or reactant, as by causing a color change within the zones (**16**, **16.1** and so on) may be poured into the upper end of the column. As the test solution flows downwardly through the column, predetermined amounts of the analyte or its derivative are reacted with or bound to the reactant in each such layer until the analyte or its derivative have been exhausted from the solution. The concentration of analyte in the solution can be determined by merely counting the number of successive zones, beginning at the top of the column, that have changed color. In another embodiment, the reactant that is bound to

the medium in the zones (16, 16.1 and so on) may be deactivated or disenabled by reaction with the analyte or analyte derivative or both, and the detector which is employed may determine, as by a color change, the presence of nondisabled reactant. In this embodiment, the reactant in successive zones contacted by the test solution will be disabled until analyte and analyte derivative have been exhausted from the solution. Upon detecting which of the reaction zones contain reactant that has not been disabled, one may determine the concentration of analyte in the solution by counting the number of zones beginning at the top of the column in which reactant is not detected. Of course, in this embodiment as in the embodiment set out above, one may also count the number of zones beginning with the bottom of the column as well.

Another physical embodiment of the apparatus of the invention is shown in FIGS. 2 and 3 in which "wicking", or upward capillary flow of a liquid through a strip of filter paper or similar material is employed, the strip having successive, spaced reaction zones. In this embodiment, the permeable solid medium may take the form of a strip of filter paper, which is designated generally as (20) in FIGS. 2 and 3. Within spaced zones (20.1) of the filter paper strip is bound a reactant, as above-described, the spaced zones being separated by spacer layers or sections (20.2). One method of preparing the strip (20) involves binding a reactant to small, individual rectangular paper filter pieces, and then alternating these pieces, which form the reaction zones, with similar pieces of filter paper that do not contain the reactant, the alternating pieces of filter paper being held together, for example, by a thin strip of adhesive tape. Other, more sophisticated methods of manufacture will be apparent to those skilled in the art.

As shown in FIGS. 2 and 3, the strip (20) of filter paper may be positioned in an elongated plastic holder (22) having a generally C-shaped cross-section. The bottom of the holder is adapted to receive the end of a wick (24) which may consist of twisted strands of cotton or other fibrous material. A similar wick (24.1) is received in the upper end of the plastic holder. The ends of the wicks (24, 24.1) contact the ends of the filter strip (20). As shown in FIG. 2, the upper and lower sections of the filter paper strip which come into contact with the wicks (24, 24.1), are spacer layers (20.2) so that each reaction zone (20.1) is sandwiched between spacer layers (20.2). The filter paper strip and holder are adapted for insertion into a test tube (26) or other container so that the lower wick (24) contacts the bottom of the test tube and the upper wick (24.1) extends out of the test tube and then downwardly toward its bottom, all as shown in FIG. 2. A test solution (28) placed in the bottom of the test tube (26) is thus caused to flow by capillary action upwardly through the length of the filter paper strip, successively contacting the reaction zones (20.1) in a manner analogous to the flow of test solution through the column depicted in FIG. 1. As will be described more fully below, the filter paper strip and holder can be moved from one test tube to another so that different solutions can be caused to sequentially flow through its length.

Referring now to FIG. 4, an apparatus of the invention is embodied in a disc of permeable, solid medium such as filter paper, porous glass, etc. (30). The disc (30) may be placed horizontally in a suitable container such as a petri dish. At its center, the disc (30) is provided with a well (30.1) to receive a test solution or other solution. Reaction zones, spaced radially from the well (30.1), are shown as annular rings (30.2), and are separated from one another by spacer layers also in the form of annular rings (30.3). Spacer layers preferably form the innermost and outermost rings of the disc. The reaction zones (30.2) and the spacer layers (30.3) are concentric. Test solution that is admitted to the central well (30.1) is thus carried radially outwardly of the well by capillary action or by diffusion, aided by centrifugal force if desired, the test solution successively passing through the spaced reaction zones (30.2).

FIGS. 5 and 6 show another embodiment of a device of the invention. The device includes a filter paper strip (40) similar to that of FIGS. 2 and 3 and containing spaced reaction zones (40.1) separated by spacer layers (40.2). A holder, preferably of plastic, is designated (42) and has a flat base (42.1) with upwardly extending legs (42.2, 42.3) carried at its ends. The leg (42.2) is provided with an upwardly open well (42.4) into which may be inserted the upper end of the filter paper strip (40), care being taken that the end (42.5) of the filter paper extends downwardly to the floor of the well. The strip of filter paper extends obliquely downwardly from the well, and its lower end is captured in a slot (42.6) formed in the leg (42.3). In use, the test solution or other solution is placed in the well (42.4), and is carried downwardly of the strip by both gravity and capillary action, the solution sequentially encountering the spaced reaction zones (40.1).

FIG. 7 shows yet another embodiment of a device of the invention which can be used for multiple concurrent tests. The device, designated (50), includes a pair of spaced plates (50.1, 50.2). Referring to the right-hand portion of FIG. 7, the space between the plates is divided into generally vertical channels by means of elongated spacers (52, 52.1). As shown in the drawing, the channel (52.2) formed by the spacers has a wide upper section and a narrow lower section. The lower section is provided with a series of vertically spaced reaction zones (54) comprising a liquid-permeable solid medium to which is bound a reactant, the medium being any of those described above. Between the reaction zones, are placed spacer layers (56), the spacer layers sandwiching between them the reaction zones (54). Between the spacers (52, 52.1), at the upper end of the channel is placed an elongated vertical divider (52.3) which divides the upper portion of the channel into two sections (52.4) and (52.5). A plug (58), which may be made of the same material as the spacers, has an upper, finger-gripping portion (58.1) and a lower, tapering plug portion (58.2) adapted to be inserted in the channel (52.5). The flat surfaces of each of the spacers and plug, of course, contact the facing surfaces of both glass plates to prevent leakage of material from the channels.

In use, a solution such as a test solution is poured into the upper end of the channels formed by the spacers (52, 52.1), and the plug (58) is then inserted to provide an air-tight upper seal in the one channel (52.5). As a result, liquid in the other channel (52.4) preferentially flows downwardly through the reaction zones and spacer layers. When the liquid level in the channel (52.4) falls below the lower end of the spacer (52.3), air can bubble upwardly through the channel (52.5), permitting the contents of that channel to empty downwardly through the reaction zones as well. In this manner, the sequential flow of liquid, first from channel (52.4) and then from channel (52.5), is rendered automatic. Preferably, one of the plates (e.g., plate 50.1) is transparent so that the results of any color change in the reaction zones may readily be observed. The other plate (50.2) may be transparent or may be of an opaque white or other light color to serve as a background against which color changes can readily be seen.

ANALYTES-REACTANTS

Analytes that can be detected in accordance with the present invention include substantially all chemical sub-

stances that are reactive with a reactant to form a product, as above discussed. It will be understood that the invention is not limited to any particular analyte or reactant, but is useful for substantially any analyte-reactant combination.

Many analytes may be analyzed simply by adapting known chemical reactions to the invention.

For example, carbon dioxide may be analyzed with phenolphthalein at a slightly alkaline pH. Calcium ion may be analyzed utilizing a reactant comprising calmodulin and mammalian phosphodiesterase or another calmodulin-sensitive enzyme (Maekawa and Abe, *Biochemical and Biophysical Research Communications* 97:621 (1980)). Ferrous ion may be analyzed utilizing, as a reactant, a ferrocene derivative (Katz, et al, J. Am. Chem. Soc. 104:346 (1982)). A large number of additional examples may be selected from the list of organic analytical reagents compiled by John H. Yoe in *Handbook of Chemistry and Physics*, p.D 126–129, 57th Edition, Robert C. Weast, Ed., CRC Press, Cleveland, 1976, and in other references cited therein.

Typical analyte-reactant pairs selected from the field of organic chemistry similarly may be chosen by adapting known chemical reactions to the invention. For example, almost any phenol may be analyzed with Gibbs Reagent (2,6-dichloro-p-benzoquinone-4-chlorimine) (Dacre, J., Analytical Chemistry 43:589 (1971)). A reagent for Indoles is p-dimethylaminobenzaldehyde (Fieser and Fieser, *Reagents For Organic Synthesis*, Volume 1, p.273, John Wiley & Sons, Inc., New York, (1967). The last-mentioned reference also shows the use of phenylhydrazine as a reactant for cortisone and similar steroids, and the use of sulfoacetic acid as a reactant in the Liebermann-Burchard test for unsaturated sterols. Amino acids and ammonium salts may be analyzed using the reagent ninhydrin (indane-1,2,3-trione hydrate) (Pasto, et al *Organic Structure Determination*, p.429, Prentice-Hall, Inc., Englewood Cliffs, N.J., 1969). Reducing sugar may be measured with Red Tetrazolium (2,3,5-triphenyl -2H-tetrazolium Chloride) (Fieser, Organic Experiments, p.135, Raytheon Education Co., Lexington, Mass., 1968).

Various other analyte reagent pairs may be selected from the field of chemistry for adaptation to the invention from such reference works as: Schuurs, et al, U.S. Pat. No. 3,654,090 (Enzyme-Linked Immunosorbent Assay); Kay, U.S. Pat. No. 3,789,116 (Fluorescent Labeled Antibody Reagents); Rubenstein, et al, U.S. Pat. No. 3,817,837 (Homogeneous Enzyme Immunoassay); Ling, U.S. Pat. No. 3,867,517 (Radioimmunoassay); Giaever, U.S. Pat. No. 3,906,490 (Radial Immunodiffusion); Ullman, U.S. Pat. No. 3,996,345 (Flourescence Quenching Homogenous Immunoassay); Maggio, U.S. Pat. No. 4,233,402 (Enzyme Channeling Homogeneous Enzyme Immunoassay); Boguslaski, et al, Canadian Patent 1,082,577 (Hapten-Cofactor Homogeneous Enzyme Immunoassay); Schonfeld, H., Ed., "New Developments in Immunoassays", *Antibiotics and Chemotherapy*, Volume 26, 1979; O'Sullivan, et al, "Enzyme Immunoassays: A Review", *Annals of Clinical Biochemistry* 16:221 (1979); Schuurs, et al, *Enzyme Immunoassay*, Clin. Chim. Acta. 81:1 (1977); Feldmann, et al, Eds., *First International Symposium On Immunoenzymatic Techniques*, INSERM Symp. No. 2, North Holland Publishing Co., Amsterdam, 1976; Williams, et al, *Methods in Immunology and Immunochemistry*, Volume 3, Academic Press, New York, 1971; and Yalow, et al, J. Clin. Invest. 39:1157 (1960).

Yet other analyte-reactant pairs may be found in: reference works such as Feigl, F., *Spot Tests in Inorganic Analysis*, 6th edition, Elsevier Publishing Co., New York, 1972; Feigl, Fritz, *Spot Tests in Organic Analysis*, 7th edition, Elsevier Publishing Co., New York, 1966; Snell, F. and Snell, C., *Colorimetric Methods of Analysis*, Vols. 1–4AAA, Van Nostrand Reinhold Co., New York, 1967–74, and Braibanti, A., Ed. *Bioenergetics and Thermodynamics: Model Systems—Synthetic and Natural Chelates and Macrocycles as Models for Biological and Pharmaceutical Studies*, D. Reidel Publishing Co., Boston, 1980.

The references identified above are incorporated herein by reference.

Of particular importance to the instant invention are those analyte-reactant combinations that form specific binding pairs of which one is an antibody and in which the other is a ligand to which the antibody is specific.

Such immunochemical reactant pairs are well-known in the art, and a wide variety of tests have been devised to detect the presence or quantity or both of an analyte, particularly when the analyte is present only in exceedingly small concentrations. Reference is made to the above-identified patents and publications.

DETECTORS

The detectors useful in the invention are capable of detecting the presence in the successive reaction zones, of analyte, analyte derivatives, reactants, or the predetermined reaction product, all as described above. The means of detection may take various forms. In the preferred embodiment, detection is signaled by a change of color, or a lack of a change of color, in the respective reaction zones of the apparatus. However, detection may be signaled by other means as well, such as by luminescence or fluorescence of the zones, radioactivity of the zones, etc. For many reactions, detection is signaled by a change in pH, and the detector may hence take the form of a pH color indicator such as phenolphthalein, Nile Blue A, Thymol Blue, and Methyl Violet. In other tests, one may detect the presence or absence of the appropriate chemical moiety in a reaction zone by observing whether a solid reaction product has settled upon the succeeding spacer layer. Various detector mechanisms are known to the art, and need not be described in detail. In the preferred embodiment, however, which makes use of immunochemical reaction between the analyte or the analyte and its derivative and the reactant, often very small concentrations of analyte are to be measured and accordingly a magnifying or amplifying mechanism may suitably be employed. One such mechanism makes use of enzymes to promote the reaction of a reaction product with a detector moiety to in turn provide a visual color indication. For example, the analyte to be tested may be provided in admixture with a known quantity of an analyte-glucose oxidase conjugate as an analyte derivative, the spaced, sequential reaction zones of the apparatus containing an antibody specific to the analyte. A signal generating system, such as horseradish peroxidase bound to the antibody in the permeable solid medium in such zones and a chromogenic material such as o-dianisidine (added, with glucose, to the test solution) can be employed. The addition of the test solution, containing the analyte, the analyte-glucose oxidase conjugate, glucose, catalase and o-dianisidine, is then flowed through the apparatus, such as the column depicted in FIG. 1. The analyte and the analyte-glucose oxidase conjugate compete for binding sites on the bound antibody, thereby resulting in a color formation due to the reaction of the o-dianisidine with hydrogen peroxide produced by the glucose oxidase—catalyzed reaction of oxygen with glu-

cose. Unreacted analyte and analyte-glucose oxidase conjugate flow to successive zones until the admixture has been exhausted of analyte and analyte-glucose oxidase conjugate. A variety of modifications of this procedure, of course, are known to the art.

EXAMPLE I

The chromogen 5,5' [3-(2-Pyridyl)-1,2,4-triazine-5,6-diyl]bis-2-furansulfonic acid, disodium salt, ("Ferene", a trademarked product of Chemical Dynamics Corp.) is used for the determination of serum iron in soluble assays through measurement of the absorbance at 593 nm, at which wavelength any interference from other pigments in the serum should be minimized. It may be covalently coupled to useful carrier derivatives through nitration, reduction, diazotization and diazonium coupling to proteins (such as albumin) immobilized on agarose beads, paper strips or other suitable permeable solid medium. This immobilized signal-generating reagent (chromogenic chelating agent) is physically arranged in sequential spaced layers or bands (reaction zones) through which the test fluid will migrate.

Small columns are prepared from silanized Pasteur pipettes by breaking off both ends, attaching a short piece of tubing to the bottom (constricted) end and inserting glass wool plugs in the tube bottoms. The columns are packed by sequential insertion of layers of agarose-Ferene separated by layers of unmodified agarose. Typically, 0.4 ml of a 1:1 agarose suspension is applied directly above the support, followed by alternating layers of 50 microliters of a 1:1 suspension of agarose-Ferene and 0.2 ml of agarose suspension. After each addition to the column, the walls of the columns are rinsed with phosphate-buffered saline (PBS) and the solution above the gel is allowed to flow into the gel before addition of the next layer.

For use in the assay, the tubing at the bottom of the prepared column is connected to a peristaltic pump to control the flow rate of the assay. An appropriate dilution of test sample for iron analysis is applied to the column. The iron solution ("test" solution) is passed through the assay column at controlled flow rates, typically between 10 and 15 minutes for complete entrance. When all the solution is into the gel bed, the columns are rinsed with water. As the test solution flows through, color develops in some of the Ferene-containing reaction zones. The number of colored zones resulting is a function of the concentration of iron in the test solution.

EXAMPLE II

A. The enzyme cholinesterase reacts with and is inhibited by toxic organophosphate and carbamate agents. Cholinesterase and the chromogenic sulfhydryl reagent 5,5'-Dithiobis-(2-nitrobenzoic acid) (Ellman's Reagent) are immobilized upon agarose beads, which are then assembled into columns according to Example I. A test solution (diluted blood serum) is added to the column and migrates through the reaction zones following which a solution of butyrylthiocholine iodide is added. The zones retaining catalytically active cholinesterase will generate a yellow color through reaction of the thiocholine produced by the hydrolytic activity of the cholinesterase, with the immobilized Ellman's Reagent. The presence of reactive cholinesterase-inhibiting toxin in the test sample will result in fewer colored bands, which will be in the downstream end region of the column.

B. Amino acids and other nucleophilic amine compounds are measured by use of the chromogenic reagent 2,4-dinitrofluorobenzene (FDNB), which produces a yellow product upon reaction. A 0.1 ml aqueous sample, containing about 0.1–1.0 micromoles of amino analyte, is transferred to a siliconed glass vessel. The pH is adjusted to 7.0, if necessary, and two milligrams (25 micromoles) of $NaHCO_3$ is added and dissolved. Next is added 0.12 ml of 0.15% FDNB in absolute alcohol (1.5 micromoles). This solution is prepared fresh shortly before use. After the reaction has neared completion, it is analyzed for remaining FDNB (reactant) content by flow exposure to the assay system prepared according to Example I. In this case, a similar amine-containing analyte is immobilized in the zones in a permeable solid medium at a known content (e.g., 0.1–0.25 micromoles per reaction zone). After rinsing with 50% ethanol in aqueous solution, the number of yellow reaction zones produced and remaining after rinse will be inversely related to the amount of analyte in the test sample.

EXAMPLE III

The IgG fraction from rabbit anti-penicilloylbovine gamma globulin was partially purified by precipitation with 33% saturated ammonium sulfate. The precipitate was redissolved and dialyzed against phosphate buffered saline (PBS). This IgG preparation was used for immobilizing antibodies onto beaded agarose. The agarose was suspended in dioxane, then reacted with carbonyldiimidazole. After being washed with dioxane, it was suspended in water, and then in aqueous borate buffer, pH 9.0. The IgG was then added to the activated agarose and the gel suspension stirred by rocking at 4° C. for 2 days. After extensive washing with PBS, the gel containing immobilized antibody was ready for use in the assay.

Small columns were prepared from silanized Pasteur pipettes by breaking off both ends, attaching short pieces of tubing to the bottom (constricted) ends and inserting glass wool plugs in the column bottoms. The columns were packed by sequentially inserting alternating layers of agarose-IgG separated by layers of unmodified agarose. Typically 0.4 ml of 1:1 agarose suspension was applied directly above the plugs, followed by alternating layers of 50 microliters of a 1:1 suspension of agarose-IgG (to form the reaction zones) and 0.2 ml of agarose suspension (to form spacer layers). After each addition, the walls of the columns were rinsed with PBS and the solution above the gel was allowed to flow into the gel before addition of the next layer.

For use in an assay, tubing at the bottom of the prepared column was connected to a peristaltic pump to control the flow rate of the assay. An appropriate dilution of penicilloyl-glucose oxidase ("Pen-GO") (typically 0.1 microgram Pen-GO in 1 ml PBS) with or without known amounts of the analyte (penicilloyl-epsilon amino caproate) (Pen-EAC), was applied to the column. The Pen-GO was prepared by reacting penicillin G with glucose oxidase in borate buffer, pH 9.0, for 2–3 days at 4° C. The Pen-GO solution was passed through the assay column at controlled flow rates, typically between 10 and 15 minutes for complete entrance into the gel. When all the solution was into the gel bed, a detector solution was added to the column. The detector solution was prepared as follows: 0.20 ml of horseradish peroxidase (HRP) solution (2 mg/ml), 2 ml of 18% glucose solution, 1 ml of 0.2 M phosphate buffer (pH 6.0) and 0.100 ml of 1% o-dianisidine was diluted 1:10 in PBS and 1 ml or less was applied to the columns at the same flow rate as the previous solutions. Brown color developed in some of the reaction zones. Presence of the penicilloyl moiety in the Pen-Go solution results in the upper reactive zone or zones being lighter in color, with color being generated in zones further down the column.

This Example may be repeated for the analysis of serum albumin (a large protein molecule) by replacement of the penicillin-glucose oxidase conjugate with an albumin-glucose oxidase conjugate.

EXAMPLE IIIA

Peroxidase-labeled IgG prepared from rabbit antiserum against penicillin was immobilized in small strips of filter paper by the method reported in Example III. Catalase was bound to other, similar strips of filter paper. The first and second mentioned strips were then cut into rectangular shapes to provide, respectively, reaction zones and spacer layers. The small rectangular pieces of filter paper were then layed onto a strip of adhesive tape, alternating the reaction zones and spacer layers with edges of the sequential pieces of paper overlapping or at least touching one another to provide a continuous capillary flow path.

Penicilloyl-glucose oxidase ("Pen-GO") in a solution of Human Serum Albumin ("ESA") was freeze-dried inside a test tube. Within another test tube, made of brown glass for protecting the contents from light, was freeze-dried a solution of o-dianisidine and glucose in phosphate-buffered saline at pH 6.0.

A short wick was attached to the bottom of the prepared filter paper strip described above, and a longer wick was placed in contact with the upper end of the strip. The strips themselves can be stored under refrigeration, and preferably are retained in a wet condition resulting from the preparation described above.

In one example of use, a test solution consisting of a measured volume of milk containing a known concentration of penicillin G is added to the test tube containing the freeze-dried Pen-GO, and the tube is shaken gently to mix the contents. The filter paper strip is then inserted into the tube with its upper, longer wick extending over the lip of the tube and then downwardly as shown in FIG. 2. When the entire solution has been taken up by the strip (or, alternatively, when the solution reaches an arbitrary flow line marked on the upper wick and designated "F" in FIG. 2) the filter paper strip is removed from the test tube and is placed in the brown glass tube to which previously has been added water to dissolve the freeze-dried contents thereof. The latter solution similarly wicks upwardly through the filter paper strip, causing color development to occur in certain of the reaction zones as determined by the quantity of penicillin G in the initial test solution.

In this example, the penicillin G in the milk and the penicillin of the Pen-GO compete for binding sites on the antibodies immobilized in the reaction zones of the filter paper strip. Of course, larger concentrations of penicillin G in the milk sample cause the penicillin G and the Pen-GO to migrate further through the filter paper strip. The presence of Pen-GO in any of the zones is indicated through the development of color from the reaction of H_2O_2 with o-dianisidine, the H_2O_2 being formed from the glucose oxidase in the presence of glucose and oxygen, and as catalyzed by the peroxidase. The catalase in the spacer layers catalyzes the conversion of H_2O_2 into O_2 and H_2O, and thus prevents migration of H_2O_2 from one reactive zone to another.

As with each of the apparatuses described herein, the device of this example may be calibrated by determining how many of the reaction zones become colored as a result of the test procedure. For example, one of the reaction zones may change color only when the test solution (e.g., milk) contains at least 9 nanograms of analyte (e.g., penicillin G) per ml. For a sample of milk containing an unknown concentration of penicillin G, one merely counts the number of reaction zones that have changed color to find the narrow, defined concentration range within which lies the penicillin G concentration.

EXAMPLE IV

Antibody against a polyvalent antigen (e.g., serum albumin) analyte is labeled with peroxidase and bound to permeable solid medium according to Example III to form reaction zones in a column. Another batch of the same or similar antibody is labeled with an enzyme such as glucose oxidase. Into the column is poured a test sample containing an unknown amount of analyte antigen. Through the column is then flowed the soluble glucose oxidase-antibody in the presence of glucose plus catalase plus o-dianisidine. The number of colored bands resulting is directly related to the amount of analyte antigen in the test sample relative to the antigen binding capacity of the antibody zones. In this example, the antigen first reacts with the bound antibody and binds to the antibody, forming a predetermined product. The latter, in turn, is detected by the coupling of the glucose oxidase antibody conjugate to available antigenic sites on the antigen followed by the color forming reaction.

EXAMPLE V

An analyte or a derivative thereof (e.g., penicillin-peroxidase) is covalently bound to a permeable solid medium according to Example III. An enzyme-labeled receptor (e.g., glucose oxidase-antibody against penicillin) is prepared and exposed to the immobilized analyte to form the specific binding complex (e.g., immune complex). The assay unit is assembled according to Example III. Subsequent exposure to a test sample containing an unknown amount of analyte is done at elevated temperature (e.g., 60° C.) to hasten the attainment of equilibrium through competitive binding of the immobilized analyte and analyte in the test sample with the enzyme-labeled antibody. Analyte in the test sample under such conditions will competitively displace the labeled antibody from the immobilized analyte. The number of colored reaction zones resulting from the procedure is inversely related to the amount of analyte in the test sample. These bands will appear in the terminal or downstream portion of the column.

EXAMPLE VI

Three assay columns with 4 reaction zones each were prepared according to Example III, except that the top reaction zone was prepared with 75 microliters of IgG-agarose suspension (1:1) and the lower 3 zones with 50 microliters. Test samples containing 0, 50 and 200 nanograms Pen-EAC, were placed in different columns, with each test sample containing 200 ng Pen-GO per ml. Flow time for sample application was 20 min. Application of the solution of signal generating reagents produced 2 colored zones with the 0 ng Pen-EAC sample, 3 in the 50 and 4 with the 200 ng sample.

A wider and more precise range of analyte content, of course, may be measured by using a larger number of assay zones.

In a preferred embodiment, only a single pass through the apparatus of a single liquid material is required. An analyte may be mixed with an analyte derivative, chromogen or other material and flowed through the apparatus to yield an appropriate test result. In a further preferred embodiment, the apparatus is chemically complete in that it includes all

reactants and other chemicals necessary or desirable for the quantitative analysis of an analyte; that is, all that is required is that the analyte in a liquid carrier be flowed through the apparatus. Elements of the apparatus that, if combined, would undergo reaction in the absence of the analyte may be maintained in different zones. For example, the bottom-most layer (20.2) of the strip of FIG. 2 may contain a reactant physically separated from reactants in the adjacent reaction zone. When the analyte in a carrier liquid is flowed through the layer (20.2), the reactant in this layer together with the analyte and carrier liquid is flowed into the first reaction zone. If desired, a reactant may be provided in the form of a solid and may merely be placed upon the upper layer (18.4) of the column of FIG. 1, the reactant being dissolved by and carried with the liquid carrier and analyte into the column.

The above-described embodiments are typified by the following Examples VII–IX which also describe and exemplify a preferred format of the invention.

This format requires at least two enzymes, one of which is coupled to an analyte to form an analyte derivative and catalyzes a color-forming reaction, and another enzyme that is immobilized in reaction zones which also contain antibody to the analyte, the latter enzyme providing substrate for the color-generating enzyme. In this format, therefore, only a single solution which consists of or contains the analyte test sample is flowed into or through the solid medium after which color develops in the reaction zones, the number of colored zones being directly related to the concentration of analyte in the test sample.

EXAMPLE VII

The IgG fraction from rabbit anti-penicilloyl-bovine serum albumin was partially purified by precipitation with 33% saturated ammonium sulfate. This protein was coupled to microcrystalline cellulose by reaction of the cellulose with carbonyldiimidazole in dioxane, followed by washing and then by reaction with the IgG preparation in borate buffer at pH 9.0 at 4 degrees C. for two days. The cellulose was then washed extensively with PBS and used for preparation of banded strips. Glucose oxidase was also coupled to microcrystalline cellulose in the same manner. A penicilloyl-peroxidase was prepared by first coupling a polyacrylamide amine to HRP, then reacting penicillin G with that preparation. It is believed that the use of a linear polymer as a spacer for attaching the hapten to the enzyme allows more hapten molecules to be coupled to each enzyme molecule and renders the hapten molecules more accessible for binding to antibody, thus speeding the binding rate. Polyacrylamide was synthesized by dissolving 0.5 gm. of acrylamide in 200 ml. of deionized water, degassing, then adding 0.2 ml. of N,N,N',N'-tetramethylethylenediamine and 0.15 gm. of ammonium persulfate. This solution was mixed, then allowed to sit at room temperature for 30 min. then passed through an ultrafiltration membrane, dialyzed vs. deionized water and lyophilized. The polyacrylamide was then dissolved in 1.0 ml. of 0.2 M phosphate buffer at pH 7.7 and 0.3 ml. of 25% glutaraldehyde was added. This solution was incubated at 37 degrees C. for 19 hours after which it was passed through a Sephadex G-25 column to remove the excess glutaraldehyde. The void volume fractions which absorbed strongly at 230 nm were pooled and added to a solution of diaminodipropylamine (0.5 ml. in 2.0 ml. of water) at pH 9.0. This solution was allowed to react at 4 degrees C. over night. The reaction mixture was then passed through a Sephadex G-150 column and the fractions that absorbed significantly at 230 nm. were divided into four pools of equal volume, the second of which was coupled to peroxidase (ERP). HRP was reacted with 1.25% glutaraldehyde at pH 7.0 for 15 hours at room temperature. After passing the reaction mixture through a Sephadex G-25 column, the HRP-containing fractions were pooled and added to the polyacrylamide-diamine preparation, the pH was adjusted to 9.0, and this solution was allowed to react at 4 degrees C. overnight. The peroxidase-polyacrylamide-diamine was then passed through a Biogel P-100 column and the void volume fractions were pooled and concentrated, then reacted with penicillin. Fifty mg. of penicillin G was added to the peroxidase-polyacrylamide-diamine, the pH adjusted to 9.0 and stirred at 4 degrees C. over night. This preparation was then dialyzed extensively, then used for the assay.

Banded strips were prepared by cutting 0.5×8.0 cm. strips of a polyester film having a hydrophilic surface onto which were glued strips of Whatman 3MM chromatography paper. At one end was glued a 0.5×4.0 cm. long paper strip followed by a 3.5 mm. space. Then three one cm. long paper strips were glued onto the Mylar strip with 2.0 mm. spaces between them. The paper on the Mylar was wetted with a solution of 0.02% o-dianisidine in water. The spaces were then filled in with a suspension of microcrystalline cellulose prepared by mixing 50% suspensions of the IgG-cellulose and the glucose oxidase-cellulose in a 20:1 ratio. The first space was filled with 20 ul. of this suspension and the other three spaces each contained 10 ul. These strips were air dried, then stored dry until used.

The strips were developed by placing the end with the longer paper spacer into a small vial containing the developing solution. This solution contained peroxidase-polyacrylamide-diamine-penicillin (25 ul. of a 0.25 microgram/ml. solution), glucose (0.3 ml of a 1.125% glucose solution in 0.2M phosphate buffer at pH 6.0) and 10 ul. of dilutions of penicilloyl-aminocaproic acid (EAC) in water. Under these conditions, pink bands could readily be observed after 20–30 min., such that, with no penicilloyl-EAC in the developing solution, one band was colored; with 0.4 micromolar hapten (penicilloyl-EAC), two bands were colored; and with 1.0 uM penicilloyl-EAC, all three bands were colored.

If needed or desired, antibody to peroxidase, an HRP-binding lectin or some other binder or inactivator of peroxidase can be included in the spacer layers for the purpose of improving the sharpness or decisiveness of zone color determinations. Furthermore, catalase immobilized in the spacer layers may permit more rapid color development in the reaction zones without generation of color in the spacer layers.

EXAMPLE VIII

Banded strips are prepared according to Example VII, except that all of the components of the assay except the sample to be tested are incorporated into the strip. The peroxidase-polyacrylamide-diaminepenicillin is dissolved in a solution of between 0.5 and 1.0% gelatin containing 2.5% glucose and 0.2 M phosphate buffer at pH 6.0, 0.1 ml. of which is applied to the bottom paper strip and dried. In this example, therefore, the user has only to dip the strip into a solution suspected of containing the analyte, wait for a prescribed time, then read the results by counting the number of colored bands on the strip.

EXAMPLE IX

Assay columns are prepared according to Example III, except that the reaction zones are composed of a mixture of

IgG-agarose and glucose oxidase-agarose (20:1). Peroxidase-penicillin (as prepared in Example VII), glucose, o-dianisidine, and phosphate buffer, stored in dry form, are dissolved in 1.0 ml of the test sample which is then added to the column and allowed to flow through. The results are read after the prescribed time by counting the number of colored bands on the column. The reagents added to the analyte test sample can be in the form of a small pellet or can be dried onto the under surface of the cap for a small vessel used to measure the volume of sample, etc. In the latter case, the vessel is filled, the cap placed on top, the vessel inverted a few times and the sample is poured into the column. The reagents to be mixed with the sample can even be dried onto a small plug that is stored in the top of the column, in which case they dissolve when the sample is added to the column.

Various other enzyme pairs can be used for generating color in the reaction zones. For example, alkaline phosphatase can be immobilized in the reaction zones with beta-galactosidase coupled to the analyte. The use of naphthol-beta-D-galactopyranoside-6-phosphate as substrate for the alkaline phosphatase results in the generation of naphthol-beta-D-galactopyranoside, which is hydrolyzed by beta-galactosidase to produce naphthol which in the presence of a diazonium salt results in a colored product in the reaction zones.

The accuracy and reliability of the apparatus of the invention depends to some extent upon how readily or easily the generation of color or other detectable change in the different reaction zones may be ascertained. A reaction zone in the direction of analyte flow desirably should show detectable changes only when a significant, minimum quantity of analyte or other material being detected has passed through the preceeding reaction zone; since the physical nature of the apparatus often does not permit reaction to go fully to completion in each such zone, a small "tail" e.g., trace, amount of material may flow into successive zones and may be marginally detected in such zones to yield readings that are difficult to interpret. One may largely avoid this problem, however by several means. Detectors may be employed that are sensitive only to minimum concentrations of a chemical moiety to be detected. For example, one may utilize o-phenylene diamine in place of o-dianisidine as a chromophore in the above examples, the former being less sensitive. Another method involves the placement in spacer layers or, less desirably, in reaction zones, of small quantities of "scavenger" reactants capable of immobilizing or deactivating trace amounts of materials, as exemplified in Example VII. This enables the sensitivity and operation of the apparatus to be tailored as desired to particular analyses. Control of sensitivity and reliability also may depend upon the concentration of the reactant in the solid reaction zones, and the solubility of materials such as the colored product in some analyses.

While a preferred embodiment of the present invention has been described, it should be understood that various changes, adaptations and modifications may be made therein without departing from the spirit of the invention and the scope of the appended claims.

What is claimed is:

1. A device for detecting the presence of an analyte in a carrier liquid suspected of containing said analyte, which device comprises a liquid permeable solid medium which defines a path for fluid flow capable of supporting capillary flow, along which are i) a site for application of the carrier liquid, ii) a diffusively bound labeled reactant specific for the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety, said labeled reactant being capable of flow along the flow path, wherein said diffusively bound labeled reactant and said analyte or chemical moiety are of a specific ligand-anti ligand pair, and iii) one or more zones spaced along said flow path, each zone having a predetermined amount of a reactant bound to it which is specific for either the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety;

wherein said device can be used by contacting a carrier liquid with said application site in such a manner that permits said liquid to pass along the flow path by capillary flow such that analyte or reaction product of the analyte with another chemical moiety becomes bound to both the labeled reactant and the reactant bound to the solid medium; and wherein the labeled reactant, with the reactant bound to the solid medium, sandwiches the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety.

2. A device according to claim 1 wherein said labeled reactant is labeled with an enzyme and is detected by adding a substrate for said enzyme.

3. A device according to claim 1 wherein said site for application of said carrier liquid is a well into which said carrier liquid is placed.

4. A device according to claim 3 which is adapted such that said carrier liquid is drawn out of said well by the capillary action of said liquid permeable solid medium.

5. A device according to claim 1 further comprising a chemical moiety diffusively bound along said flow path, said moiety being capable of reacting with said analyte in order to form a reaction product.

6. A device according to claim 1 wherein said solid permeable medium contains all the materials needed for detecting the presence of said analyte so that all that is required is the application of said carrier liquid.

7. A method for detecting the presence of an analyte in a carrier liquid suspected of containing said analyte, which method comprises;

a) providing a liquid permeable solid medium which defines a path for fluid flow capable of supporting capillary flow, along which are i) a site for application of the carrier liquid, ii) a diffusively bound labeled reactant specific for the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety, said labeled reactant being capable of flow along the flow path, wherein said diffusively bound labeled reactant and said analyte or chemical moiety are of a specific ligand-anti ligand pair, and iii) one or more zones spaced along said flow path, each zone having a predetermined amount of a reactant bound to it which is specific for either the analyte or a chemical moiety which is itself the reaction product of the analyte with another chemical moiety;

b) contacting said application site with said carrier liquid such that the liquid passes along the flow path by capillary flow such that analyte or reaction product of the analyte with another chemical moiety becomes bound to both the labeled reactant and the reactant bound to the solid medium; and

c) detecting the labeled reactant which, together with the reactant bound to the solid medium, has sandwiched the analyte or chemical moiety as an indication of the presence of analyte.

8. A method according to claim 7 wherein said labeled reactant is labeled with an enzyme and is detected by adding a substrate for said enzyme.

9. A method according to claim 7 wherein said site for application of said carrier liquid is a well into which said carrier liquid is placed.

10. A method according to claim 9 which is adapted such that said carrier liquid is drawn out of said well by the capillary action of said liquid permeable solid medium.

11. A method according to claim 7 wherein said solid permeable medium contains all the materials needed for detecting the presence of said analyte so that all that is required is the application of said carrier liquid.

12. A method according to claim 7 wherein said carrier liquid passes as a single material in a single pass.

* * * * *

EXHIBIT C

US006534320B2

(12) **United States Patent**
Ching et al.

(10) **Patent No.: US 6,534,320 B2**
(45) **Date of Patent: *Mar. 18, 2003**

(54) **PROCESS FOR IMMUNOCHROMATOGRAPHY WITH COLLOIDAL PARTICLES**

(75) Inventors: **Shanfun Ching**, Libertyville, IL (US); **Patricia A. Billing**, Gurnee, IL (US); **Julian Gordon**, Lake Bluff, IL (US)

(73) Assignee: **Abbott Laboratories**, Abbott Park, IL (US)

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/046,171**

(22) Filed: **Mar. 23, 1998**

(65) **Prior Publication Data**

US 2001/0006821 A1 Jul. 5, 2001

Related U.S. Application Data

(63) Continuation of application No. 08/543,227, filed on Oct. 13, 1995, now abandoned, which is a continuation of application No. 08/260,612, filed on Jun. 16, 1994, which is a continuation of application No. 07/809,598, filed on Dec. 18, 1991, which is a continuation of application No. 07/072,459, filed on Jul. 13, 1987, now Pat. No. 5,120,643.

(51) **Int. Cl.**[7] **G01N 33/559**

(52) **U.S. Cl.** **436/514**; 436/506; 436/518; 436/524; 436/525; 435/7.92; 435/5

(58) **Field of Search** 436/514, 506, 436/518, 524, 525; 435/5, 7.92

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Primary Examiner—Lynette R. F. Smith
Assistant Examiner—Ginny Allen Portner
(74) *Attorney, Agent, or Firm*—Regina M. Anderson

(57) **ABSTRACT**

The present invention relates to improved specific binding assay methods, kits and devices utilizing chromatographically mobile specific binding reagents labelled with colloidal particles. Specific binding reagents labelled with colloidal particles such as gold and selenium may be subjected to rapid chromatographic solvent transport on chromatographic media by means of selected solvents and chromatographic transport facilitating agents. Further, impregnation of solid substrate materials with labile protein materials including colloidal particle and enzyme labelled reagents in the presence of meta-soluble proteins provides for the rapid resolubilization of such materials which have been dried onto such substrate materials.

13 Claims, 3 Drawing Sheets

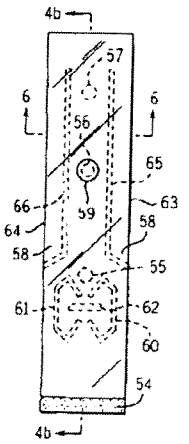

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FIG. 1a

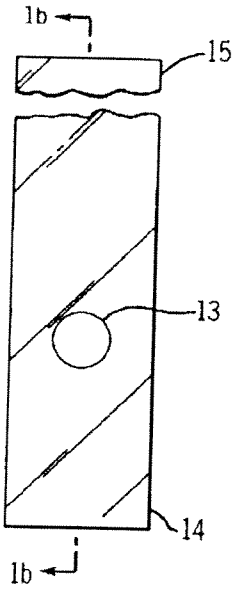


FIG. 1b

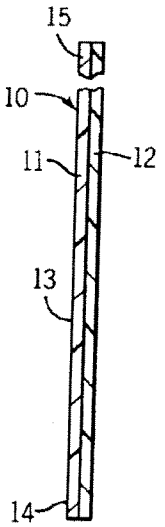


FIG. 1c

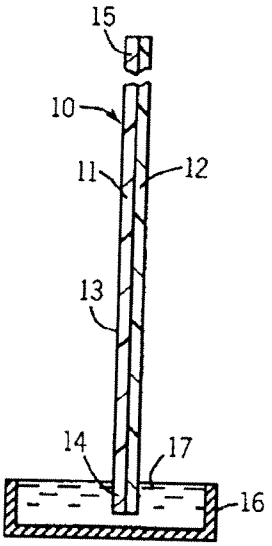


FIG. 2a

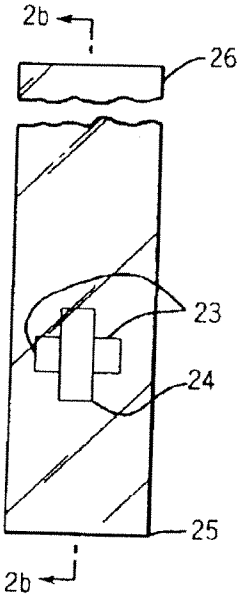
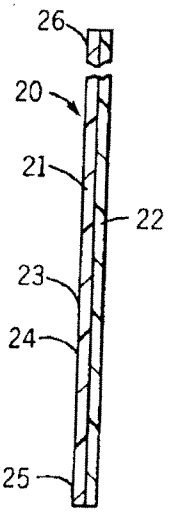


FIG. 2b



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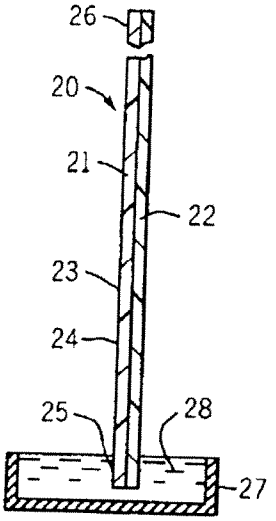


FIG. 3a

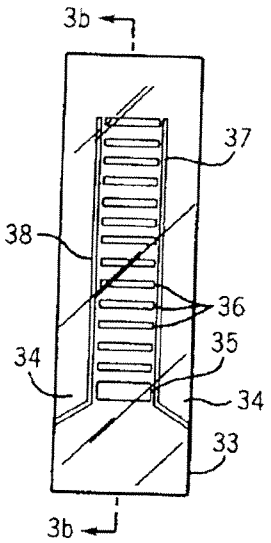


FIG. 3b

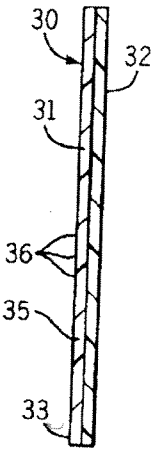


FIG. 3c

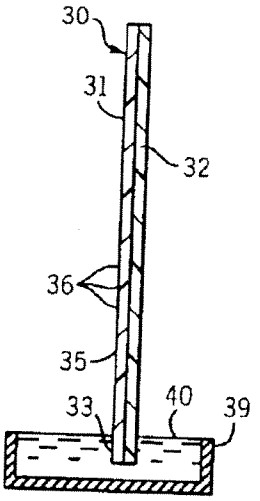


FIG. 4a

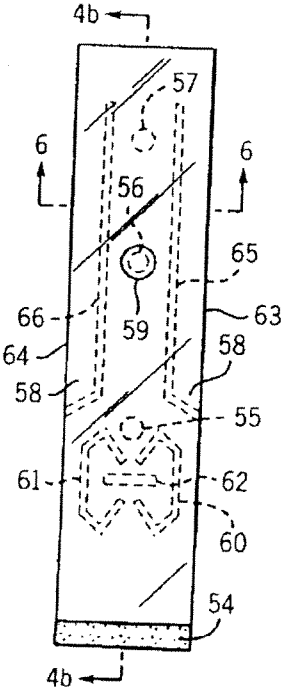


FIG. 4b

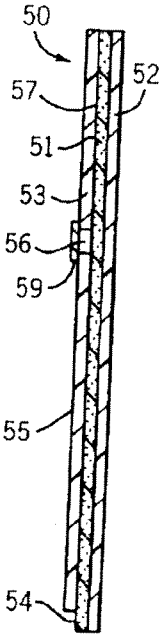


FIG. 4c

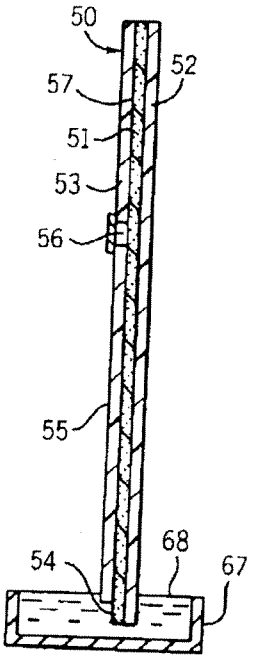


FIG. 5a

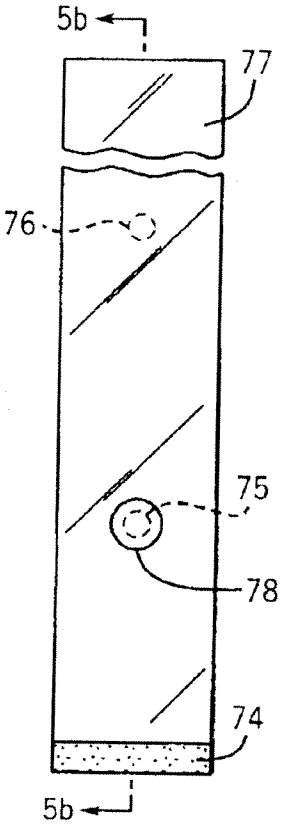


FIG. 5b

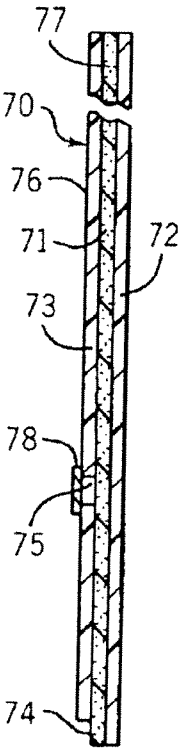
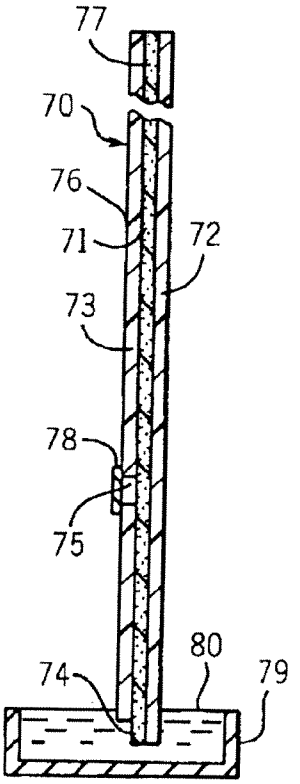


FIG. 5c



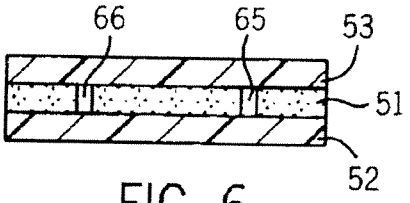


FIG. 6

PROCESS FOR IMMUNOCHROMATOGRAPHY WITH COLLOIDAL PARTICLES

The present application is a continuation of U.S. Ser. No. 08/543,227, filed on Oct. 13, 1995, now abandoned which is a continuation of U.S. Ser. No. 08/260,612, filed on Jun. 16, 1994, which is a continuation of U.S. Ser. No. 07/809,598, filed on Dec. 18, 1991, which is a continuation of U.S. Ser. No. 07/072,459, filed on Jul. 13, 1987, now U.S. Pat. No. 5,120,643.

BACKGROUND

The present invention relates generally to assay devices and specifically to those devices making use of chromatographic techniques in conducting specific binding assays. According to one aspect of the invention, methods and devices are provided utilizing colloidal particle labelled specific binding materials which are chromatographically mobile and capable of producing visually detectable signals. According to another aspect of the invention, methods and devices are provided utilizing labelled specific binding materials including colloidal particle labelled materials and enzyme labelled materials which are dried onto a chromatographic medium in the presence of a meta-soluble protein and are capable of being rapidly resolubilized in the presence of an appropriate solvent such as the sample or a chromatographic transport solvent.

Immunological assays have proven to be of great value in a variety of clinical applications. Such assays depend upon specific binding reactions between immunoglobulins (antibodies) and materials presenting specific antigenic determinants (antigens). Antibodies bind selectively with ligand materials presenting the antigen for which they are specifically reactive and are capable of distinguishing the ligand from other materials having similar characteristics.

Because the results of immunological and other specific binding reactions are frequently not directly observable, various techniques have been devised for their indirect observation. Such techniques involve labelling of one of the members of the specific binding pair with a radioisotope, chromophore, fluorophore or enzyme label. Radiolabels, chromophores and fluorophores may be detected by the use of radiation detectors, spectrophotometers or the naked eye. Where members of a specific binding pair are tagged with an enzyme label, their presence may be detected by the enzymatic activation of a reaction system wherein a compound such as a dyestuff, is activated to produce a detectable signal.

There are three well known types of immunological specific binding assays. In competitive binding assays, labelled reagents and unlabelled analyte compounds compete for binding sites on a binding material. After an incubation period, unbound materials are washed off and the amount of labelled reagent bound to the site is compared to reference amounts for a determination of the analyte concentration in the sample solution. A second type of immunological assay is known as a sandwich assay and generally involves contacting an analyte sample solution to a surface presenting a first binding material immunologically specific for that analyte. After a wash step, a solution comprising a labelled second binding material specifically reactive with the analyte to be detected is then added to the assay. The labelled second binding material will bind to any analyte which is itself bound to the first binding material. The assay system is then subjected to a wash step to remove any labelled second binding material which failed to bind with

the analyte. The amount of labelled material remaining may then be determined and will be indicative of the amount of analyte present in the sample. While the term sandwich assay is frequently understood to relate to immunological assays wherein the first and the labelled reagent materials are both antibodies or are both antigens such that the "sandwich" is of the form antibody/antigen/labelled antibody, a broader definition of the term sandwich-type assay is understood as including other types of three component assays including what are sometimes referred to as "indirect sandwiches", which may be of the form antigen/antibody/labelled (anti-immunoglobulin) antibody.

A third type of immunological assay is the agglutination assay which is exemplified by well-known assays for blood antigens and serum types. Immunological reactivity between antibodies within serum and antigens presented on red blood cell surfaces is indicated by the formation of a three dimensional cross-linked network of antigen (red blood cells) and antibodies. The agglutination of the serum/red blood cell mixture results in the formation of a macroscopic pellet in the testing well which can be visible to the naked eye.

These various immunoassay procedures were originally performed as "liquid phase" assays in apparatus such as test tubes where antigen/antibody conjugates were centrifuged and precipitated. More recently, methods have been developed wherein antibodies or antigens are coated onto the surface of microtiter wells and reactions are carried out in solution in such wells. Methods have also been developed for carrying out "solid phase" assays wherein immunological reactions are carried out in solution on solid substrates including those which are porous or fibrous materials. According to such procedures, porous carrier materials are fashioned into strips or other forms to which antibodies or antigens are immobilized by adsorption, absorption or covalent bonding. Sample materials containing an analyte specifically reactive with the immobilized member of the binding pair are applied to the carrier material where the analyte is immobilized by reaction with its corresponding binding pair member. The non-reacted sample materials are then removed by a washing step after which, in the case of a sandwich-type assay, a labelled reagent is applied to the carrier material which is capable of reaction with and immobilization by the immobilized analyte. The carrier material is then washed in order that the presence of the labelled reagent, and hence the analyte, may be detected.

Modifications of such "solid phase" assays are known wherein one or more of the sample components or reagents is moved by means of chromatographic solvent transport. U.S. Pat. No. 4,168,146 to Grubb, et al., discloses porous test strips to which antibodies have been immobilized. The strips are then contacted with measured amounts of aqueous solution containing the analyte antigen. Antigen molecules within the test solution migrate by capillary action throughout the test strip, but because the bound antibodies retard the migration of the antigens for which they are specific, the extent of migration of the antigen molecules over a fixed time period is a function of the antigen concentration in the test solution. The antigen-containing areas of the diagnostic device are then indicated by the addition of enzyme or fluorescent chromophore labelled antibodies.

U.S. Pat. No. 4,517,288 to Giegel, et al. discloses methods for conducting solid phase immunoassays on inert porous materials. The patent discloses immunologically immobilizing a binding material within a specified zone of the porous material and applying the sample to the zone containing the immobilized binding material. An enzyme labelled indicator material which will bind with the analyte is then applied to

the zone where it will become immobilized in an amount correlated to the amount of analyte in the zone. A solvent is then applied to the center of the zone to chromatographically remove the unbound labelled indicator from the zone so that the amount of labelled indicator remaining in the zone may be measured.

Of interest to the present invention are the disclosures of the Deutsch, et al., U.S. Pat. Nos. 4,094,647, 4,235,601 and 4,361,537 which relate to immunological and other types of specific binding assays wherein reagents are transported by chromatographic solvent transport. According to one embodiment, a radiolabelled competitive binding assay kit comprises a strip capable of transporting a developing liquid by capillarity having a first zone for receiving a sample, a second zone impregnated with a first reagent capable of being transported by the developing liquid and a third zone impregnated with a second reagent. In addition, the devices comprise a measuring zone and a retarding element which may be either the second reagent or the material of the strip. The first reagent is capable of reacting with one of the group consisting of (1) the sample, (2) the sample and the second reagent, or (3) the second reagent in competition with the sample, to form a product in an amount dependent on the characteristic being determined. A sample is contacted with the first zone and the strip is then dipped into the developing liquid to bring about transport of the sample and the first reagent to form the reaction product. The retarding element slows transport of either the product or the first reagent (the moving reagent) to spacially separate the two and the amount of the moving element is then measured at the measurement location.

The Deutsch, et al., patents relate to methods wherein reagents located on the chromatographic material are mixed with the sample material and other reagents during the course of chromatographic transport. Such mixing is not detrimental to and may even be desirable for competitive binding assays. It may, however, be undesirable for sandwich-type binding assays where it is necessary to prevent contact between non-analyte sample materials and labelled specific binding reagents.

Of interest to the present invention is the disclosure of U.S. Pat. No. 4,452,901 to Gordon which relates to the use of porous nitrocellulose supports for immobilization of proteins. It is disclosed that such nitrocellulose sheets may be utilized in immunoassay procedures if the residual binding capacities of the nitrocellulose sheets are saturated by blocking treatment with one or more types of proteins, different from those immobilized and not cross-reactive with any of the antibodies subsequently used in the assay.

Of further interest to the background of the invention are the disclosures of Gordon, EPO Application 63,810, published Nov. 3, 1982, relating to devices for conducting immunological assays. The devices consist of a porous solid support containing a preselected array of delimited adsorption areas of antigens, antibodies or both, wherein residual adsorption sites on the substrate are saturated by protein blocking agents such as bovine serum albumin. Porous solid supports are selected from a variety of natural and synthetic polymers and derivatives but are preferably nitrocellulose sheets 0.1 mm thick with pore size between about 0.15 μm and about 15 μm. Antigens or antibodies are applied to the porous solid support by direct contact followed by incubation with blocking agents. Assays for detection of unknown antigens or antibodies are then carried out through use of labelled antibodies which may also be anti-immunoglobulin antibodies.

Also of particular interest to the present application is the disclosure of co-owned U.S. Pat. No. 4,960,691, filed Sep.

29, 1986 by Gordon, et al., which is hereby incorporated by reference and which relates to devices for conducting specific binding assays utilizing the sequential chromatographic transport of analyte and reagent materials. Wash and addition steps are inherently carried out and liquid "microcircuitry" can be programmed to carry out a variety of multistep procedures and to avoid the premature mixing of sample materials and reagents. Preferred blocking solutions for treatment of the strip materials include include 1% I.B gelatin (Inotech, Wohlen, Switzerland) in TBS solution comprising (0.15 M NaCl, 0.02 Tris-HCl, pH 7.6) or 3% bovine serum albumin (BSA) solution in physiological saline.

Specifically, the Gordon, et al., sequential transport application relates to devices which comprise a test strip for the detection of an analyte in a sample comprising a length of chromatographic material having the capacity for rapid chromatographic solvent transport of non-immobilized reagents and reactive sample components by means of a selected chromatographic solvent. The strip includes a first end at which chromatographic transport begins, a second end at which chromatographic transport ends and a plurality of zones positioned between the two ends. The zones include a first zone (impregnated with a first reagent which is mobile in the solvent and capable of reaction with, and immobilization against solvent transport by the analyte when the analyte is in immobilized form), a second zone (for receiving the sample suspected of containing an analyte) and a third zone (positioned downstream of the first zone and impregnated with a second reagent which is immobilized against solvent transport and is capable of selective reaction with the analyte so as to render the analyte in an immobilized form in the third zone). The device is further characterized in that after the sample is received in the second zone and upon the first end being dipped into the chromatographic solvent, the relative mobility of the analyte and the first reagent or the site relationship between the second and third zones is such that the analyte is disposed and immobilized against solvent transport at the third zone prior to the first reagent reaching the third zone, whereby interfering sample components and non-analyte components of the sample which are reactive with the first reagent are cleared from the third zone by chromatographic solvent transport prior to transport of the first reagent to the third zone. The presence of the first reagent immobilized at the third zone may be detected by means of enzyme, radioisotope or other labels. The device is particularly suited for use with enzyme labelled reagents as enzyme substrates and indicator dye reagents may be incorporated on separate zones on the strip and transported to the third zone in an appropriate sequence by chromatographic transport.

Of interest to the present invention are those references relating to the use of dispersions of colloidal particles in immunological assay procedures. Frens, Nature, 241, 20–23 (1973) discloses methods for the preparation of monodisperse gold sols of various particle sizes through the reduction of gold chloride with aqueous sodium citrate. Variation in the concentration of sodium citrate during the nucleation of the particles may be used to vary the particle size of the resulting sols. Sols of mono-dispersed gold particles are disclosed having particle sizes ranging from 16 nm to about 150 nm and exhibiting colors ranging from orange to red to violet over that range.

Romano, et al., Immunochemistry, 11, 521–22 (1974) discloses the labeling of immunoglobulins with colloidal gold particles for use in imaging human red blood cell antigens by means of electron microscopy. The gold sol,

which has an average particle diameter of about 3 nm, has a tendency to flocculate but is stabilized by the presence of either horse serum or BSA.

Geoghegan, et al., J. Immuno. Meth., 34, 11–21 (1980) discloses the coating of colloidal gold particles with immunoglobulins for use in passive agglutination procedures. The reference (at page 14) discloses the resuspension of centrifuged pools of gold labelled immunoglobulins with 0.01 M phosphate buffered saline (PBS) (pH 7.2) containing 1% polyethylene glycol (PEG). The reference also notes that while the gold-protein complexes do not aggregate during centrifugation, they are often subject to non-specific aggregation in the presence of any serially diluted protein in a microtiter plate.

Surek, et al., Biochem. and Biophys. Res. Comm., 121, 284–289 (1984) discloses the use of protein A labelled colloidal gold particles for the detection of specific antigens immobilized on nitrocellulose membranes. According to the procedure, an electrophoresis gel is blotted onto a nitrocellulose filter which is then treated with a 2% solution of BSA in PBS to prevent non-specific binding. The filter is treated with diluted antiserum or preimmune serum and washed with PBS-BSA. The strip is then incubated for 30 to 60 minutes with protein A conjugated with colloidal gold which detects the presence of bound antibodies. Excess unbound colloidal gold particles are then removed by several short buffer washes.

Leuvering, U.S. Pat. No. 4,313,734 discloses the use of metal sol particles as labels for in vitro determination of immunological components in an aqueous test medium. Specifically disclosed are immunoassay test kits for the detection of antigens or antibodies employing one or more labelled components obtained by coupling the component to particles of an aqueous sol dispersion of a metal, metal compound or polymer nuclei coated with a metal or metal compound having a particle size of at least 5 nm. According to one example, an assay for human placental lactogen (HPL) is conducted with the use of rabbit anti-HPL antibodies which have been labelled with gold particles. Unlabelled rabbit anti-HPL antibodies are coated onto the walls of microtiter plate wells by incubation with BSA solution and phosphate buffer to which merthiolate has been added. Standard solutions of HPL are added to the wells and were incubated for 2 hours at room temperature. A solution consisting of rabbit anti-HPL antibodies which has been conjugated with gold particles having diameters between 45 and 70 nm is added to the wells and incubated at room temperature overnight. The wells are then washed and light absorption measured with a small-volume spectrophotometer.

Hsu, Anal. Biochem. 142, 221–225 (1984) discloses the use of immunogold marker systems for blot immunoassay procedures wherein serial dilutions of purified tobacco mosaic virus (TMV) are electrophoresed in a polyacrylamide gel and are then electrotransferred to nitrocellulose filter sheets. The nitrocellulose sheets are baked to stabilize binding and treated with 5% normal goat serum or in 0.05% TWEEN 20 (polyoxyethylene sorbitanmonolaurate) in PBS to block nonspecific antibody binding. The filter is incubated overnight at 4° C. with rabbit anti-TMV antibodies diluted in blocking solution followed by washing in PBS and the antigen-antibody complex is then detected by soaking the filter in gold-labelled goat anti-rabbit IgG in blocking solution. According to the procedure as little as 8 ng of the TMV protein is detectable with about 30 minutes exposure to the gold-labelled IgG. The reference also discloses that agents such as polyethylene glycol, polyvinylpyrrolidone and bovine serum albumin can enhance the stability of gold markers. The use of TWEEN 20 (polyoxyethylene sorbitanmonolaurate) to prevent nonspecific binding of protein on nitrocellulose is disclosed along with the observation that 0.05% TWEEN 20 (polyoxyethylene sorbitanmonolaurate) in PBS can be used in the staining procedure without disturbing the specificity of the gold-IgG complexes. Normal goat serum is identified as a preferred blocking agent in light of its tendency to adsorb gold particles that may dissociate from the probe during storage.

Moeremans, et al., EPO Application No. 158,746 discloses the use of colloidal metal particles as labels in sandwich blot overlay assays. Specific binding materials specifically reactive with the analyte to be detected are applied to nitrocellulose strips and dried. Protein binding sites on the strip are then blocked by means of treatment with bovine serum albumin, gelatin, polyethylene glycol or polyoxyethylene sorbitanmonolaurate (TWEEN 20). Analyte containing sample material is then applied to the strip and incubated for 2 hours. The treated strip is washed and air-dried and incubated for 2 hours with a specific binding agent which has been labelled with colloidal metal particles. According to one example, anti-tubulin antibodies are detected by gold particle labelled reagents producing a pink-reddish color (20 nm particles) or a purplish color (40 nm particles). The assays are disclosed to have a sensitivity on the order of 5 ng/μl.

Of interest to the present invention is the disclosure of Hoye J. Chromatog., 28, 379–384 (1967) relating to chromatographic purification of radiochemicals. The application of paper chromatography, thin layer chromatography and high voltage electrophoresis techniques are disclosed to move gold ions with varying degrees of success but are generally unsuitable for transporting colloidal gold particles.

The use of polymerized dye materials in colloidal form for specific binding assays is also known. Of interest to the present application is the disclosure of Hirschfeld, U.S. Pat. No. 4,166,105 which relates to labelled specific binding reagents reactive with specific antigens prepared by linking fluorescent dye molecules to analyte specific antibodies through polymers comprising reactive functional groups. Also of interest to the present application is the disclosure of Henry, U.S. Pat. No. 4,452,886 which relates to specific binding reagents comprising antigens or antibodies linked to a water-soluble polymer consisting essentially of between 40 and 600 chromophoric or fluorescent group containing monomers.

SUMMARY OF THE INVENTION

The present invention provides improved specific binding assay methods, kits and devices utilizing chromatographically mobile labelled materials. According to one aspect of the invention, methods and devices are provided utilizing colloidal particle labelled specific binding materials which are chromatographically mobile and capable of producing visually detectable signals. According to another aspect of the invention, methods and devices are provided utilizing labelled specific binding materials including colloidal particle labelled materials and enzyme labelled materials which are dried onto a chromatographic medium in the presence of a meta-soluble protein and are capable of being rapidly resolubilized in the presence of an appropriate solvent such as the sample or a chromatographic transport solvent.

It has been discovered that specific binding materials labelled with colloidal particles such as gold may be subjected to rapid chromatographic transport on a chromato-

graphic medium by means of selected solvents and chromatographic transport facilitating agents and that use of chromatographic solvent transport assay techniques significantly reduces the time required for the binding reaction of a colloidal particle labelled material with its specific binding partner as compared with conventional methods. It has also been discovered that impregnation of solid substrate materials with labile proteins including labelled and unlabelled materials in the presence of an aqueous medium containing meta-soluble proteins such as casein allows the rapid resolubilization of such proteins which have been dried onto such substrate materials. Such resolubilized labelled materials may then be maintained in a liquid solution for carrying out liquid phase assays or electron microscopy. The drying of labile proteins in the presence of meta-soluble proteins thus provides an inexpensive means of storing such proteins, including colloidal particle labelled reagents, in a stable and convenient form from which they might then be resolubilized and subjected to chromatographic solvent transport. Alternatively, where the solid substrate material is a chromatographic medium, the labile proteins may be solubilized and chromatographically transported to carry out specific binding assay procedures.

Accordingly, the invention provides improved specific binding assay devices, kits and methods for determining the presence or amount of a substance in a sample. The colloidal particle labelled assays provide a visually detectable signal and do not require the use of materials such as radioisotopes or enzyme labels with the attendant requirement for detection equipment or addition of enzyme conjugates and indicator dyes. Means for conducting both competitive binding and direct binding (sandwich-type) assays are provided by the invention. Preferred assay methods and kits are provided for determining the presence or amount of a substance in a sample whereby a colloidal particle labelled material and a chromatographic transport facilitating agent are mixed with the sample. A chromatographic medium comprising one or more reaction sites impregnated with one or more reagents useful for carrying out the assay is then contacted with the mixture of sample, colloidal particle labelled material and chromatographic transport facilitating agent in order to chromatographically transport the sample and labelled material along the chromatographic medium and carry out the desired assay.

The invention also provides assay methods and devices wherein labelled materials including colloidal particle labelled materials and enzyme labelled materials are impregnated and dried onto a reaction site on a chromatographic substrate material in the presence of meta-soluble proteins. The chromatographic mediums of the devices may comprise one or more additional reaction sites where chemical reactions per se need not take place but where additional materials may be deposited or immobilized or where analyte substance containing sample materials may be deposited. The chromatographic medium is contacted with a chromatographic solvent which solubilizes and transports along the medium the colloidal particle labelled specific binding material as well as the sample substance and other optional materials and reagents. The affinity of the immobilized specific binding reagent is such that it efficiently captures the labelled material in the flowing material such that the labelled binding component is accumulated in the zone. Where the labelled material is a colloidal particle labelled material, an important advantage is provided by the present invention in that the binding affinity of the immobilized reagent may be such that it is capable of capturing a labelled component in the flowing chromatographic stream in such a way that the labelled binding component is accumulated in the capture zone and is clearly discernable over the background stream of non-concentrated colloidal particle labelled material.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1***a*, **2***a*, **3***a*, **4***a* and **5***a* are front plan views of five different forms of the test devices of the present invention;

FIGS. **1***b*, **2***b*, **3***b*, **4***b* and **5***b* are cross-sectional views of the test devices shown in FIGS. **1***a*, **2***a*, **3***a*, **4***a* and **5***a*, respectively, taken along lines **1***b*—**1***b*, **2***b*—**2***b*, **3***b*—**3***b*, **4***b*—**4***b* and **5***b*—**5***b*;

FIGS. **1***c*, **2***c* and **3***c* are cross-sectional views of the test devices shown in FIGS. **1***a*, **2***a* and **3***a*, respectively, in contact with a volume of sample material and indicator solution;

FIGS. **4***c* and **5***c* are cross-sectional views of the test devices shown in FIGS. **4***a* and **5***a*, respectively, in contact with a volume of chromatographic solvent; and

FIG. **6** is a cross-sectional view of the test device of FIG. **4***a* taken along lines **6**—**6**.

DETAILED DESCRIPTION

The present invention provides improved immunological and other specific binding assay methods, kits and devices utilizing chromatographically mobile labelled specific binding materials.

Colloidal particle labelled specific binding materials are highly susceptible to aggregation and are thus generally incapable of being rapidly and efficiently transported on chromatographic media according to chromatographic solvent transport assay methods. The invention is based on the discovery that specific binding materials labelled with colloidal particles, and particularly with colloidal particles larger than about 1 nm in diameter, which are especially subject to aggregation, may be subjected to rapid chromatographic transport on chromatographic media by means of selected solvents and chromatographic transport facilitating agents. While materials labelled with colloidal particles and particularly those less than about 1 nm in diameter may be capable of chromatographic transport without the presence of the chromatographic transport facilitating agents of the present invention, the use of such agents assists in the rapid chromatographic transport of all colloidal particle labelled reagents of the invention. As a related discovery, it has been found that chromatographic solvent transport of colloidal particle labelled materials significantly reduces the time required for the binding reaction of those materials with their specific binding partners as compared with conventional methods. While conventional immunoassay procedures such as those of Leuvering, U.S. Pat. No. 4,313,734 teach the incubation of colloidal particle labelled specific binding materials for from 1 hour to overnight (16 hours), the chromatographic methods of the present invention generally provide for the rapid completion of transport and specific binding reactions in less than about 5, and preferably less than about 2, minutes.

As another aspect of the present invention, it has furthermore been discovered that labile protein materials including colloidal particle, enzyme or other labelled materials which are impregnated and dried onto solid substrate materials in the presence of selected meta-soluble protein materials may be rapidly solubilized by means of suitable solvents. Where the solid substrate material is a chromatographic medium, the labelled materials may be rapidly resolubilized and

transported along the chromatographic medium by means of selected chromatographic solvents. As a consequence of this discovery, specific binding assay devices are provided wherein labelled materials including colloidal particle labelled specific binding materials are incorporated in dry stable form on the device and only the sample material and a chromatographic solvent need be added for conducting an assay.

According to the invention, kits may be produced and specific binding assay methods may be practiced for analysis of a substance in a sample according to a method employing a solution comprising a colloidal particle labelled material. The method also employs a chromatographic medium having capillarity and the capacity for chromatographic solvent transport of non-immobilized reagents and reactive sample components by means of a selected chromatographic solvent including a reaction site including an immobilized reagent capable of binding a member selected from the group consisting of the substance to be analyzed and the colloidal particle labelled material. The method comprises (a) contacting the sample to be analyzed to the chromatographic medium, (b) chromatographically transporting on said chromatographic medium said colloidal particle labelled material whereby at least a portion of said colloidal particle labelled material is chromatographically transported to the reaction site for binding thereto, and (c) determining the detectable response produced by said colloidal material at the reaction site as an indication of the presence or amount of the substance in the sample.

According to preferred embodiments of the invention, the affinity of the immobilized reagent and the concentrations of reagents and sample materials may be selected by one of skill in the art such that the colloidal particle labelled material is accumulated at the reaction site and is detectable over the background stream of the non-concentrated colloidal particle labeled material. Where this is not the case, the chromatographic medium may be subjected to a wash or rinse step to remove the unbound labelled material. Such wash steps may also be inherently carried out according to the procedures of co-owned U.S. Pat. No. 4,960,691.

It is also to be understood that said sample to be analyzed, said colloidal particle labelled material, said chromatographic solvent and other solvents and reagents may be mixed with each other according to various possible combinations prior to their contacting the chromatographic medium and that these mixtures and various components may be contacted to the chromatographic medium in various sequences as would be apparent according to the skill in the art. It is to be further understood that the chromatographic solvent may be replaced by the sample or by the solution containing the colloidal particle labelled material where these materials are capable of transporting the colloidal particle labelled material to the reaction site at which the reagent is immobilized. It is also to be understood that the chromatographic solvent may inherently be used according to the methods of co-owned U.S. Pat. No. 4,960,691 to wash unreacted labelled materials and other non-immobilized sample components from the reaction site at which the reagent is immobilized. When the labelled material is contacted to the chromatographic medium at the reaction site at which the reagent is disposed, the chromatographic solvent transport may be used to accelerate the binding reaction between the colloidal particle labelled material and other specific binding reagents as well as wash non-immobilized labelled material from the zone.

A preferred embodiment of the invention is that wherein the indicator solution additionally comprises a chromatographic transport facilitating agent and it and the sample are mixed and contacted to the chromatographic medium to provide for chromatographic transport of the analyte substance and the labelled specific binding material. According to other embodiments, the sample material and the colloidal particle labelled material may be contacted to one or more reaction sites on the assay device upstream of the reaction site at which the reagent is immobilized and the chromatographic medium is contacted with chromatographic solvent to transport the sample and the labelled material to the reaction site at which the second reagent is immobilized.

Sandwich-type assays may be practiced according to the method wherein the colloidal particle labelled material is capable of participating in a specific binding reaction with the analyte substance. Competitive binding assay methods may also be practiced wherein the colloidal particle labelled material is capable of participating in a specific binding reaction with the immobilized reagent. Kits may be produced and the method may also be practiced wherein the chromatographic medium comprises a second reaction site impregnated with a second reagent which is immobilized against solvent transport and is capable of selective reaction with the colloidal particle labelled material to render it in an immobilized form in the second reaction site where it may be detected. In sandwich-type assays, the presence of colloidal particle labelled material at the second reaction site acts as a control and confirms the reactivity of the first reagent. In competitive binding assays, the presence of colloidal particle labelled material at the second reaction site indicates the degree of competition between the analyte substance and the immobilized reagent.

Other methods and devices are provided according to the invention wherein labelled specific binding materials including, but not limited to, colloidal particle labelled and enzyme labelled materials are incorporated on the chromatographic medium of an assay device in a dry form which may be rapidly resolubilized and chromatographically transported along the medium by selected chromatographic solvents. Such specific binding assay devices comprise a chromatographic medium having capillarity and the capacity for chromatographic solvent transport of one or more non-immobilized reagents and reactive sample components by means of a selected chromatographic solvent. The devices also comprise a first reaction site impregnated with a dried solution of a labelled material in the presence of a metasoluble protein wherein the labelled material is capable of rapid solubilization and chromatographic solvent transport in the solvent and a second reaction site at which is immobilized a reagent capable of binding with a member selected from the group consisting of the analyte substance and the labelled material. The device may be used by (a) contacting the sample with the chromatographic medium; (b) solubilizing the labelled material and chromatographically transporting at least a portion of the labelled material to the second reaction site for binding thereto; and (c) determining the detectable response produced by said labelled material at the second reaction site as an indication of the presence or amount of the substance in the sample.

It is to be understood that the sample to be analyzed may be mixed with the chromatographic solvent and that the chromatographic medium may be contacted to the mixture of both. It is to be further understood that the chromatographic solvent may be replaced by said sample, said sample being capable of solubilizing said immobilized first reagent and chromatographically transporting itself and said labelled first reagent to the second zone at which the second reagent is disposed.

A preferred embodiment of the present invention is that wherein the chromatographic solvent is replaced by the sample which is capable of solubilizing the dried labelled material and transporting the analyte substance and the labelled material to the second reaction site containing the immobilized reagent.

Sandwich-type assays may be practiced according to the method wherein the labelled material is capable of participating in a specific binding reaction with the analyte substance. Competitive binding assay methods may also be practiced wherein the labelled material is capable of participating in a specific binding reaction with the immobilized reagent. Devices may be produced and methods may also be practiced wherein the chromatographic medium comprises a third reaction site impregnated with a second reagent which is immobilized against solvent transport and is capable of selective reaction with the labelled material to render the labelled material in an immobilized form at the third reaction site where it can be detected. In sandwich-type assays, the presence of labelled material at the third reaction site acts as a control and confirms the reactivity of the labelled material. In competitive binding assays, the presence of labelled material at the third reaction site indicates the degree of competition between the analyte substance and the labelled reagent.

The invention also provides a method for the stable storage of labile proteins including antibodies, antigens, and enzyme labelled and colloidal particle labelled specific binding materials wherein the protein materials may be rapidly solubilized by application of a suitable solvent. The method comprises drying the protein material, preferably under a stream of air, on a substrate in the presence of an aqueous medium containing a meta-soluble protein. The resulting product comprises a solid substrate upon which is impregnated and dried a labile protein in the presence of an aqueous medium containing a meta-soluble protein. The substrate may generally be any solid material such as glass or plastic but is preferably a porous or fibrous matrix such as paper or nitrocellulose. The solid substrate may be in various forms such as strips, pellets or the wall of a test tube or microtiter well. The aqueous solution containing the meta-soluble protein, preferably casein, may optionally comprise chromatographic transport facilitating agents such as polyethylene glycol, gelatin, bovine serum albumin and detergents.

Mix and Run Assay Devices (Sandwich-Type)

According to one aspect of the present invention, specific binding assays may be conducted according to mix and run techniques wherein an indicator solution comprising a colloidal particle labelled first specific binding reagent dissolved in a chromatographic transport facilitating agent is mixed with the analyte substance containing sample. Assay devices according to the invention are then dipped in the mixture of sample and indicator solution which is chromatographically transported to a first zone where a second reagent has been immobilized. According to one embodiment of a sandwich-type assay procedure, the first and second reagents are capable of specific binding with the analyte. In this embodiment, the labelled first reagent binds with the analyte after they have been mixed. The conjugate of the labelled first reagent and the analyte is then subjected to being immobilized by reaction with the second reagent and producing a visually detectable signal at the first zone. In the absence of analyte, labelled first reagent will not bind at the zone and no signal will be produced there. Alternative sandwich-type assay procedures may be followed wherein a third reagent which is specifically reactive with the labelled first reagent is immobilized at a second zone to provide a control. Still other, competitive-type assay methods and devices are provided where the immobilized second reagent is specifically reactive with both the analyte and the labelled first reagent which compete for binding with the immobilized reagent.

Referring to the drawing, FIGS. **1***a*, **1***b* and **1***c* depict a test device (**10**) for the detection of an analyte in a sample liquid comprising a length of chromatographic substrate material (**11**) with a first end (**14**) at which chromatographic solvent transport begins, a second end (**15**) at which chromatographic solvent transport ends and a first zone (**13**) impregnated with a second reagent which is immobilized against solvent transport and is capable of selective reaction with the analyte so as to render the analyte in an immobilized form. The device further comprises an inert support strip (**12**) to which the length of chromatographic material (**11**) is affixed.

According to a procedure for use of the device (**10**), a quantity of the sample to be tested is mixed with an indicator solution comprising a colloidal particle labelled first reagent and a chromatographic transport facilitating agent. The quantity and concentration of the chromatographic transport facilitating agent in the indicator solution added to the sample is selected such that it prevents aggregation and provides for rapid chromatographic solvent transport of the colloidal particle labelled specific binding reagent. In sandwich-type mix and run assays, the labelled first reagent will react-to specifically bind with the analyte. The test device (**10**) is then dipped at its first end (**14**) into a container (**16**) containing the mixture of sample and indicator solution (**17**) and the sample/indicator solution mixture containing the labelled first reagent/analyte conjugate progresses through the chromatographic material (**11**) to the first zone (**13**). The second specific binding reagent immobilized at the first zone (**13**) is also specifically reactive with the analyte and will react with the analyte or with first reagent/analyte conjugate to immobilize it at the first zone (**13**). The chromatographic solvent transport is such, however, that labelled first reagent which is not conjugated with the analyte along with other sample and indicator solution materials which are not immobilized at the first zone (**13**) are transported away from that zone. Chromatographic solvent transport continues until the sample/indicator solution mixture is depleted or until the sample/indicator solution front reaches the second end (**15**) of the device.

Analyte present in a sample will bind with the labelled first reagent and will be chromatographically transported to the first zone (**13**) where it will be immobilized by a specific binding reaction with the second reagent. Where sufficient analyte is present in a sample, the number of colloidal particles thus immobilized at the first zone (**13**) will be such as to produce a visually detectable signal. Of course, if no analyte is present in the sample, neither analyte nor labelled first reagent will be immobilized at the first zone, and no signal will be produced.

Mix and Run Assay Devices (Competition-Type)

The device (**10**) according to FIG. **1** may also be modified to perform competition-type specific binding assays. Specifically, the colloidal particle labelled first reagent may be selected to compete with the analyte for binding with the immobilized second reagent. Referring to the drawing, FIG. **1** depicts a test device for performing mix and run competition-type assays. The device itself and the identity of the immobilized second reagent is the same in the competition-type assay as in the sandwich-type assay. The only difference in the assay kits lies in the identity of the colloidal particle labelled first reagent material. In the

sandwich-type assay kits of the invention, the labelled first reagent is specifically reactive with the analyte while in competition-type assays the labelled first reagent is a specific binding analogue of the analyte to be assayed and is specifically reactive with the immobilized second reagent in competition with the analyte.

According to a procedure for use of the device (10), a quantity of the sample to be tested is mixed with an indicator solution comprising a colloidal particle labelled first reagent in the presence of a chromatographic transport facilitating agent. The test device (10) is then dipped at its first end (14) into a container (16) filled with the mixture of sample and indicator solution (17). The sample/indicator solution mixture containing the labelled first reagent and the analyte progresses through the chromatographic substrate material (11) to the first zone (13). The labelled first reagent and the analyte then compete to bind with the second specific binding reagent immobilized at the first zone (13). The chromatographic solvent transport is such, however, that analyte and the colloidal particle labelled first reagent materials which do not bind specifically with the immobilized second reagent are removed from the first zone (13) by the chromatographic solvent. Chromatographic solvent transport will continue until the quantity of sample/indicator solution is depleted or until the solution front reaches the second end (15) of the device.

The quantity of analyte present in the sample will determine the amount of labelled first reagent which binds at the first zone (13). Adjustments of the quantity and/or binding affinity of the labelled first reagent can be made in order to determine the quantity of analyte present in the sample.

Alternative Mix and Run (Sandwich-Assay)

According to another aspect of the present invention, assay devices and kits are provided for performing sandwich-type assays which comprise a control function and which provide a positive or negative signal for the detection of a particular analyte. Referring to the drawing, FIG. 2 depicts a test device (20) comprising a length of chromatographic substrate material (21) with a first end (25) at which chromatographic solvent transport begins, and a second end (26) at which chromatographic solvent transport ends. The device also comprises a first zone (23) which may be separated and broken up into two or more areas and which is impregnated with a second reagent which is immobilized against solvent transport and is capable of selective reaction with the analyte so as to render the analyte in an immobilized form. The device (20) also comprises a second zone (24) which is impregnated with a third reagent which is immobilized against solvent transport and is capable of selective reaction with the colloidal particle labelled first reagent.

The first and second zones (23) and (24) may be shaped such as to provide a signal of distinctive shape when one but not the other or when both comprise an immobilized reagent. For example, the shapes of the first and second zones (23) and (24) are such in FIG. 2 that when label is immobilized at the second zone (24) only a minus (−) sign is indicated. When, on the other hand, label is immobilized at both the first (23) and second (24) zones a plus (+) sign is indicated.

It is preferred that the first zone impregnated with a reagent specifically reactive with the analyte to be detected be oriented essentially perpendicular to the direction of chromatographic flow. This is because the analyte and hence the labelled specific binding reagent tend to become immobilized at the leading rather than the trailing edge of the zone. Where an elongated zone is oriented with its major dimension parallel to the direction of chromatographic flow, the leading portion of the zone will trap a majority of the analyte with the result that the trailing end traps little analyte and the resulting visible signal has a shape which may be misinterpreted. By orienting the first zone perpendicular to the direction of chromatographic flow, a stronger and more distinct positive detection signal is produced.

According to a procedure for use of the device (20) a quantity of the sample to be tested is mixed with an indicator solution comprising a colloidal particle labelled first reagent in the presence of a chromatographic transport facilitating agent. The test device (20) is then dipped at its first end (25) into a container (27) containing a mixture of sample and indicator solution (28) and the sample/indicator solution containing the labelled first reagent/analyte complex progresses through the chromatographic material (21) to the first (23) and second (24) zones. The immobilized second reagent material at the first zone (23) is specifically reactive with the analyte and will immobilize the analyte as well as any analyte/labelled first reagent complex. In addition, the immobilized third reagent material at the second zone (24) is specifically reactive with the labelled first reagent and will immobilize the first reagent as well as any analyte/labelled first reagent conjugate.

Where analyte is present in the sample, analyte/labelled first reagent conjugate will form in the mixture of the indicator solution and sample and the conjugate will be immobilized at both the first (23) and second (24) zones thus, according to one embodiment, producing a plus (+) sign and positive signal. Where no analyte or less than a threshold amount is present in the sample, the first reagent will react with the third reagent at the second zone (24) and will be immobilized at that zone producing a visual signal. Because no analyte is present, nothing will be immobilized at the first zone (23) and no signal will be produced and only a minus (−) sign will appear indicating the absence of analyte. The presence of the signal at the second zone (24) but not the first (23) in addition to indicating absence of analyte will indicate the mobility of the labelled first reagent and will serve as a control relating to the utility of the assay device.

Alternative Mix and Run (Competitor-Type Assay)

According to another aspect of the present invention, assay devices and kits are provided for performing competition-type mix and run assays which comprise a first zone impregnated with a second reagent specifically reactive with the analyte to be detected and the colloidal particle labelled first reagent and one or more second zones impregnated with a third reagent specifically reactive with the labelled first reagent and not with the analyte.

Referring to the drawing, FIGS. 3*a*, 3*b* and 3*c* depict a test device (30) for the detection of an analyte in a sample liquid (40). The device (30) comprises a length of chromatographic substrate material (31) with a first end (33) at which chromatographic solvent transport begins and a second end (34), (which is not necessarily the second physical end of the strip) at which chromatographic solvent transport ends. The device (30) further comprises a first zone (35) impregnated with a second reagent which is immobilized against solvent transport and is capable of selective reaction with a member of the group consisting of the analyte and a colloidal particle labelled first reagent. Downstream of the first zone (35) is located the second zone (36) which may optionally comprise more than one area and which is impregnated with a third reagent which is immobilized against solvent transport and is specifically reactive with the labelled first reagent but not with the analyte. The device also comprises a right-hand solvent barrier means (37) and a left-hand solvent barrier

means (38) which focus the chromatographic flow of material from the first end (33) to the first (35) and second (36) zones. The solvent barrier means (37) and (38) also effectively lengthen the chromatographic substrate material (31) by providing extended chromatographic transport pathways to the second end (34).

According to a procedure for use of the device (30), a quantity of the sample to be tested is mixed with an indicator solution comprising a colloidal particle labelled first reagent in the presence of a chromatographic transport facilitating agent. The first reagent is a specific binding analogue of the analyte to be assayed and is specifically reactive with the immobilized second reagent at the first zone (35). The test device (30) is then dipped at its first end (33) into a container (39) filled with the mixture (40) of sample and indicator solution. The sample/indicator solution mixture containing the labelled first reagent and the analyte progresses through the chromatographic material (31) to the first zone (35). The colloidal particle labelled first reagent and the analyte then compete to bind with the second specific binding reagent immobilized at the first zone (35). The chromatographic solvent transport is such, however, that analyte and labelled first reagent materials which do not bind specifically with the immobilized second reagent are removed from the first zone (35) by the chromatographic solvent and are transported toward the second end (34) and to the second zone(s) (36). The labelled first reagent, which can be a mouse anti-second reagent antibody when the analyte to be detected is a human anti-second reagent antibody, then reacts with the third reagent (which can be anti-mouse IgG antibodies) immobilized at the second zone (36) which is specifically reactive with the labelled first reagent but not the analyte. The third reagent reacts with the labelled first reagent immobilizing it at the second zone (36) producing a detectable signal. The device may optionally comprise additional "second zones" such that where an excess of labelled first reagent is mixed with the sample material and the labelled first reagent is partially or wholly displaced from binding at the first zone (35), the degree of displacement may be determined from the extent of binding at the second zone(s) (36). Chromatographic solvent transport will continue and the sample/indicator solution will progress through the device until the quantity of sample/indicator solution is depleted or until the solution front progresses around the right-hand (37) and left-hand (38) solvent barrier means and reaches the second end (34) of the device.

Pre-Impregnated Labelled Specific Binding Material Devices

An alternative aspect of the present invention relates to specific binding assay devices wherein labelled specific binding reagents including those with colloidal particle or enzyme labels which are capable of chromatographic solvent transport are impregnated and dried onto the chromatographic substrate materials of the devices. It has surprisingly been found that drying of the labelled specific binding reagent materials in the presence of meta-soluble proteins, such as casein, not only provides for the rapid chromatographic solvent transport of labelled materials, but also provides for the rapid resolubilization of such labelled materials impregnated and dried onto the chromatographic substrate materials. The labelled reagents thus resolubilized are capable of being efficiently transported by means of conventional chromatographic solvent systems and of reacting in the specific binding assays.

The ability to impregnate chromatographic substrate materials with the labelled specific binding reagents, which may then be resolubilized, makes possible the practice of a variety of assay procedures which avoid the use of labelled reagent addition steps. Both sandwich-type and competition-type assays may be conducted using the kits and strips of the present invention.

Sandwich Assay Device

Referring to the drawing, FIGS. 4*a*, 4*b* and 4*c* depict a test device (50) for the detection of an analyte in a sample wherein a labelled first reagent is impregnated and dried onto the device (50). The device (50) comprises a length of chromatographic substrate material (51) with a first end (54) at which chromatographic solvent transport begins and second ends (58) at which chromatographic solvent transport ends. The length of material (51) comprises a first zone (55), a second zone (56) and a third zone (57).

Between the first end (54) and the first zone (55) is a delaying box defined by a right-forward solvent barrier means (60), a left-forward solvent barrier means (61) and a transverse solvent barrier means (62). The right-forward solvent barrier means (60) and the right edge (63) of the material define a right-hand chromatographic solvent transport pathway, and the left-forward solvent barrier means (61) and the left edge (64) of the material define a left-hand chromatographic solvent transport pathway. The right-hand and left-hand chromatographic solvent transport pathways meet downstream of the first zone (55) and the delaying box to form a center chromatographic transport pathway in which are located said second (56) and third (57) zones defined by a right-rearward solvent barrier means (65) and a left-rearward solvent barrier means (66). Downstream of the third zone (57), the right-rearward solvent barrier means (65) and the right edge (63) and the left-rearward solvent barrier means (66) and the left edge (64) define chromatographic solvent transport pathways leading to second ends (58) at which chromatographic solvent transport ends.

The first zone (55) is impregnated with a labelled first specific binding reagent which is mobile in a chromatographic solvent (68) and is capable of reaction with and immobilization against solvent transport by the analyte when the analyte is in immobilized form. The second zone (56) is downstream of the first zone (55) and provides a suitable site for receiving the sample to be analyzed. The third zone (57) is downstream of the second zone (56) and is impregnated with a second reagent which is immobilized against solvent transport and is capable of selective reaction with the analyte so as to render the analyte in an immobilized form. The device further comprises an inert support strip (52) to which the length of chromatographic substrate material (51) is affixed. The device additionally comprises a cover plate (53) which may optionally be transparent and may be placed over the length of the chromatographic material (51) leaving exposed the first end (54) of the material. The cover plate (53) defines an opening corresponding to and leaving exposed the second zone (56). A removable tab (59) covers the second zone (56).

According to a procedure for use of device (50) of FIGS. 4*a*, 4*b* and 4*c*, the first tab (59) is removed from the device (50), a sample of the material to be tested is applied to the second zone (56) and the removable tab (59) is replaced. The device (50) is then contacted at its first end (54) into a container (67) of chromatographic solvent (68). The chromatographic solvent (48) then progresses through the length of chromatographic material passing along the right-hand and left-hand chromatographic solvent transport pathways to the center chromatographic transport pathway. Some of the solvent is transported upward toward the second zone (56) while some of the solvent is transported downward toward the first zone (55) solubilizing the labelled first

reagent. A portion of the chromatographic solvent (68) from the first end (54) passes between the right-forward solvent barrier means (60) and left-forward solvent barrier means (61) into the delaying box. The chromatographic solvent passes around the transverse solvent barrier means (62) which delays its flow before it is transported toward the first zone (55). The labelled first reagent at the first zone has already been solubilized by solvent from the right and left-hand solvent transport pathways and the solvent progressing through the delaying box upon reaching the solubilized first reagent starts to transport the first reagent toward the second zone (56). The chromatographic solvent (68) which was transported through the right-hand and left-hand solvent transport pathways then contacts the sample applied to the second zone (56) and transports the sample to the third zone (57). There, the immobilized second reagent material selectively reacts with analyte present in the sample so as to immobilize it. Non-analyte components of the sample are transported away from the third zone (57). The labelled first reagent is then transported to the third zone (57) where it is immobilized against solvent transport by the analyte when any analyte is in immobilized form. Chromatographic solvent transport of the analyte-depleted sample and first reagent continues until the chromatographic solvent (68) reaches the second end (58) of the material.

A variety of sandwich-type assay devices including dried labelled reagents and preferably including dried enzyme labelled and colloidal particle labelled reagents may be produced according to the invention. It is frequently desirable to avoid premature contact of analyte and sample materials with the reagents and contact of the reagents with each other. Thus, the relative mobility of the sample components and the various reagents or the site relationship between the zones may be selected such that the reagents and sample components mix at only the times and locations desired. Co-owned U.S. Pat. No. 4,960,691 discloses various methods and devices for conducting chromatographic solvent transport assays where it is desired to avoid contact of a labelled first reagent material (such as an anti-human immunoglobulin antibody) with sample material (such as serum) prior to the time at which the analyte antibody is immobilized against solvent transport at a reaction zone and other non-analyte antibodies contained in the serum sample are cleared from the third zone by chromatographic solvent transport. The use of acceleration and delay pathways can also be particularly useful in preventing drying of sample materials or other reagents.

Competition Assay Device

The assay devices of the present invention comprising solubilizable specific binding reagents are also suitable for the practice of competitive binding type assays. According to such methods, the immobilized second reagent is selected, as in sandwich-type assays, so as to specifically bind with the analyte of interest. The labelled first reagent, however, is selected to be a specific binding analogue of the analyte which will bind competitively with the immobilized second reagent. In carrying out competition type assays according to the invention, it is generally not necessary that the analyte and the colloidal particle labelled reagent be prevented from contacting each other prior to their contacting the immobilized second reagent. Thus, the device may be designed so as to mix the analyte containing sample and the labelled first reagent. Of course, if so desired, the device may be designed so as to prevent contact of the sample and labelled first reagents until after their contacting the immobilized second reagent.

Referring to the drawing, FIGS. *5a*, *5b* and *5c* depict a test device (70) for conducting competitive binding assays for detection of an analyte in a sample wherein a labelled first reagent is impregnated and dried onto the device (70). The device (70) comprises a length of chromatographic substrate material (71) with a first end (74) at which chromatographic solvent transport begins and a second end (77) at which chromatographic solvent transport ends. The length of material (71) comprises a first zone (75) and a second zone (76). The first zone is impregnated with a labelled first reagent in the presence of a meta-soluble protein containing anti-aggregation buffer. The second zone (76) is downstream of the first zone (75) and is impregnated with a second reagent which is capable of a selective binding reaction with both the analyte and the labelled first reagent so as to render the analyte and labelled first reagent in immobilized form. The device further comprises an inert support strip (72) to which the length of chromatographic substrate material (71) is affixed. The device additionally comprises a cover plate (73) which is placed over the length of the chromatographic substrate material (71) leaving exposed the first end (74) of the material. The cover plate (73) defines an opening corresponding to and leaving exposed the first zone (75) which is covered by a removable tab (78).

According to a procedure for use of device (70) of FIGS. *5a*, *5b* and *5c*, the tab (78) is removed from the device (70), a sample of the material to be tested is applied to the first zone (75) and the tab (78) is replaced. The device (70) is then contacted at its first end (74) into a container (79) of chromatographic solvent (80). The chromatographic solvent (80) then progresses through the length of the chromatographic substrate material (71) transporting the labelled first reagent impregnated at the first zone (75) and the sample deposited there to the second zone (76). There the analyte and labelled first reagent compete to bind with the immobilized second reagent for which they are both specifically reactive. Non-analyte components as well as unbound analyte and first reagent material are transported away from the second zone (76) by means of the chromatographic solvent transport which continues until the chromatographic solvent is exhausted or the solvent front reaches the second end (77) of the material. At the conclusion of the chromatographic solvent transport, the second zone (76) may be observed to determine the presence of labelled first reagent immobilized at that location. The presence of labelled first reagent at that location may then be related to the presence of analyte in the sample. Where the first reagent is labelled with colloidal particles, its presence at the second zone may be observed directly. Where the first reagent is labelled with an enzyme label, other reagents such as enzyme substrates and indicator dyes may be added to the second zone to visualize the presence of the first reagent.

Description of the Colloidal Particles

The present invention is directed to means for improving the chromatographic transport characteristics of colloidal particles used as labels in specific binding assays. The colloidal particles that may be used in conjunction with the methods, kits and devices of the present invention are those which may be used with specific binding assays generally. Particularly well known is the use of colloidal metal particles and especially colloidal gold for carrying out immunoassays. Other colloidal particles such as polymerized dye particles which may also be used as labels in specific assay methods such as those of Hirschfeld, U.S. Pat. Nos. 4,166,105 and Henry, 4,452,886, the disclosures of which are hereby incorporated by reference, may now be used in chromatographic transport specific binding assays. Particularly preferred colloidal particle labels for use with the present invention include non-metal particles such as

selenium, tellurium and sulfur with selenium being particularly preferred according to co-owned U.S. Pat. No. 4,954,452, the disclosure of which is hereby incorporated by reference.

Colloidal particles which are suitable as labels according to the invention include those which may be conjugated to specific binding reagents without interfering with the activity of such reagents or with other reagents or analytes. The particles must be detectable and preferably produce a visually detectable signal when present in relatively low concentrations. Particles ranging in size from about 1 nm to about 200 nm in diameter are generally suitable although both larger and smaller particles are also suitable for use according to the invention. The methods of the invention are particularly useful with particles larger than about 1 nm in diameter which are particularly susceptible to aggregation. Particles larger than about 200 nm tend to exhibit diminished mobility and may tend to drop out of suspension even in the presence of the chromatographic transport facilitating agents of the present invention. Particles much larger may also have their transport limited by the pore size of the chromatographic transport material. Particles smaller than about 1 nm tend to exhibit superior chromatographic mobility to larger particles and in some cases may not require the use of the chromatographic transport facilitating agents of the present invention. Nevertheless, particles smaller than about 1 nm tend to provide weaker signals and are thus less suitable for use in assay procedures.

Colloidal metal particles are particularly suitable as labels according to the present invention and include those particles which are comprised of metals or metal compounds including metal oxides, metal hydroxides or metal salts. Such particles generally vary in diameter from about 1 nm to about 200 nm with particles ranging in diameter from about 40 nm to about 80 nm being particularly preferred. Particles may comprise pure metal or metal compounds but may also comprise polymer nuclei coated with metal or metal compounds. Such particles are disclosed to have properties similar to those of particles comprising pure metal or metal compounds. Suitable metals and metal compounds include those selected from the group consisting of the metals platinum, gold, silver and copper and the metal compounds, silver iodide, silver bromide, copper hydroxide, iron oxide, iron hydroxide or hydrous oxide, aluminum hydroxide, or hydrous oxide, chromium hydroxide or hydrous hydroxide, lead sulfide, mercury sulphide, barium sulphate and titanium dioxide. Preferred metal particles include those made up of gold silver or iron oxide.

Colloidal metal particles may be produced according to methods generally known in the art. Specifically, Frens, Nature, 241, 20 (1973) the disclosure of which is hereby incorporated by reference discloses methods for the production of gold sol particles of varying sizes. Gold particles may be produced by methods wherein a solution of gold chloride is heated to boiling and is then mixed with a solution of sodium citrate to reduce the gold chloride. Soon after mixing of the two solutions the boiling solution turns a faint blue indicating the onset of nucleation soon thereafter the blue color changes to red indicating the formation of monodisperse particles. Reduction of the gold chloride is complete after only a few more minutes of boiling. The resulting particle sizes may be controlled by variation of the concentration of the sodium citrate solution. Particles comprising other metals and metal compounds as well as particles comprising polymer nuclei may be obtained by similar methodologies. The colors of the visually detectable signal from the metal particle label is dependent upon the identity and particle size of the metal particle. For example, colloidal gold particles produce colors varying from orange to red to violet depending upon the particle size of the sol.

Non-metal colloidal particles such as those of selenium, tellurium and sulfur may be produced according to the methods of co-owned U.S. Pat. No. 4,954,452.

Conjugation of Binding Reagents with the Colloidal Particles

The specific binding reagents of the invention may be conjugated with colloidal particle labels according to methods generally known in the art. According to one general procedure, proteinaceous specific binding reagents and colloidal particles are rapidly mixed together and are incubated in a solution to which an agent such as bovine serum albumin or polyethylene glycol is added. The suspension is centrifuged first at low speed so as to remove any large aggregates and then at high speed to produce a pellet of the reagent/colloidal particle conjugate before the supernatant is aspirated and removed. The pellet is resuspended in a solution containing a chromatographic transport facilitating agent according to the invention.

The colloidal particle labels need not be conjugated directly to the specific binding reagents but may be coated or pretreated with other reagents. Leuvering, U.S. Pat. No. 4,313,734, the disclosure of which is hereby incorporated by reference, discloses methods by which metal sol particles may be coated with inert polymer and copolymer coatings. The metal sol may be brought into contact with the polymer or the sol can be placed in an environment containing one or more monomers and a polymerization reaction initiated. After coating with the inert polymer the immunological specific binding component may be coupled to the coating material by adsorption or covalent binding.

Description of the Meta-Soluble Proteins

As used herein, the term meta-soluble protein refers to those proteins which, in their native form, are hydrophobic and poorly soluble in water but which when subjected to chemical treatment, as by alkaline purification treatment, can be made more hydrophilic and thus capable of forming uniform solutions or dispersions in water. Such chemical treatments serve to cleave hydrophobic fatty acid groups from the protein molecules by cleavage of ester or other linkages. This cleavage leaves carboxy and hydroxy residues on the molecule, rendering the protein more hydrophilic at those sites. Without intending to be limited to a single theory of the invention, it is believed that alkaline treatment renders the meta-soluble proteins somewhat detergent-like, that is, presenting both hydrophobic and hydrophilic aspects. Chemical treatment, while required for practice of the invention, need not be alkaline treatment but may also be with acids, detergents or solvents such as alcohol or urea.

The proteins, when so treated, are capable of functioning as potent chromatographic transport facilitating agents thus preventing aggregation and inactivation of labile proteins and reagents such as enzyme and colloidal particle labelled reagents. In addition, the treated meta-soluble proteins when dried with a labile protein material, provides for the stable storage and prevents aggregation and inactivation when maintained in a dry state while allowing the protein materials to be rapidly resolubilized and utilized in chromatographic transport assays if so desired. The labile protein materials include antibodies, antigens or other specific binding proteins including such proteins labelled with enzymes, colloidal particles or other labels. Preferred meta-soluble proteins include materials such as casein, zein and a non-albumin component of egg white protein with casein in

concentrations of from 1 to 5% being particularly preferred for use with the invention. Preferred materials include vitamin free casein (Sigma Chemical Co., St. Louis, Mo., catalogue No. C-3400), Zein (Sigma, catalogue No. Z-3625) and egg white protein (Sigma, catalogue No. A-5253) which comprises both the meta-soluble protein responsible for solubilization and transport and the inactive albumin fractions. It is known that the egg white component responsible for solubilization and transport is not egg white albumin as the pure albumin material does not promote resolubilization and transport.

Description of the Chromatographic Transport Facilitating Agents

As used herein, the term chromatographic transport facilitating agents refers to those materials which prevent aggregation and inactivation of specific binding materials and reagents in solution and, further, which promote their chromatographic transport. The agents may be liquids or may be solids, in which case they are preferably dissolved in a solution such as a buffer salt solution. Suitable chromatographic transport facilitating agents include materials such as polyethylene glycol, proteinaceous materials such as gelatin and bovine serum albumin and detergents such as sodium dodecyl sulfate (SDS), sodium deoxycholate (DOC) and polyethylene glycol tert-octylphenyl ether (TRITON X-100). Particularly preferred is the use of meta-soluble protein materials such as casein. Meta-soluble proteins may also be used to impregnate and dry labelled reagents onto solid substrate materials including chromatographic substrate materials in such a manner that the labelled reagents may be rapidly resolubilized and transported, if desired, by means of chromatographic solvent transport.

Where the chromatographic transport facilitating agent is to be mixed with the labelled material and utilized as a component of an indicator solution with mix and run kits, it preferably comprises casein or another treated meta-soluble protein in combination with other chromatographic transport facilitating agents such as PEG with buffer salt solution. A particularly preferred chromatographic transport facilitating buffer comprises 2% casein in combination with 0.1% PEG in PBS. The concentration of the components of the buffer and of the indicator solution are selected in the practice of the mix and run kits of the invention such that for a given sample size sufficient concentrations of the components are provided to prevent aggregation and inactivation of the labelled reagents and promote their chromatographic solvent transport.

Colloidal particle labelled specific binding reagents in the presence of casein containing solutions can have Rf values approaching 1.0 while colloidal labelled materials in buffers containing PEG can have Rf values approaching 0.7. Casein concentrations in suitable chromatographic transport facilitating buffers range from between about 0.1% (w/v) to greater than about 5% with concentrations of about 2% being preferred. It is noted that concentrations greater than about 5% do not appear to assist the anti-aggregation or chromatographic transport facilitating qualities of the buffer while they may, however, tend to interfere with resolubilization of labelled reagents dried onto the test strips of the invention.

Solutions comprising PEG as the only chromatographic transport facilitating agent are suitable for practice of some aspects of the present invention. PEG containing buffers have Rfs as high as 0.7. Preferred PEG concentrations in suitable chromatographic transport facilitating buffers range from about 0.05% to about 2% with about 1% being preferred. Suitable PEG polymers may have a variety of molecular weights, with molecular weights of about 20,000 being particularly preferred.

Gelatin is generally unsuitable for use alone as a chromatographic transport facilitating agent as solutions containing it provide for an Rf of only about 0.2. It may nevertheless be useful when combined with other anti-aggregation materials of the invention. Gelatin is generally unsuitable, however, when used in concentrations greater than about 2% as it contributes to the tendency to aggregate.

Buffer solutions suitable for use with the chromatographic transport facilitating agents of the invention should have a pH between about 5 and 9 and should not interfere with the reactivity of the analyte or reagents or their chromatographic transport. Preferred buffer solutions have pHs of about 7 and include buffers such as Tris and PBS.

Description of the Chromatographic Media

Chromatographic media useful with the present invention include those chromatographic substrate materials having capillarity and the capacity for chromatographic solvent transport of non-immobilized reagents and reactive sample components by means of a selected chromatographic solvent. The chromatographic substrate materials used with the invention are preferably in the form of strips, but it is contemplated that they may be in other forms including, but not limited to, particles or gel materials in a chromatographic column. While a wide variety of chromatographic strip materials such as woven and non-woven fibrous materials used for paper chromatography are suitable for use with the invention, the use of microporous or microgranular thin layer chromatography substrates is particularly preferred as the use of such substrates improves the speed and resolution of the assays according to the invention. The materials should preferably be inert and generally not react physically or chemically with any of the sample components, reagents, colloidal particle labels, buffers or reaction products.

Thin layer chromatographic substrate materials particularly suitable for use with the present invention include granular thin layer chromatographic materials such as silica or microgranular cellulose. Preferred non-granular microporous materials include microporous cellulose esters, for example, esters of cellulose with an aliphatic carboxylic acid, such as an alkane carboxylic acid, having from 1 to 7 carbon atoms, e.g., acetic acid, propionic acid, or any of the butyric acids or valeric acids. Especially preferred are microporous materials made from nitrocellulose, by which term any nitric acid ester of cellulose is intended. Suitable materials include nitrocellulose in combination with any of the said carboxylic acid cellulose esters. Thus, pure nitrocellulose esters can be used as consisting of an ester of cellulose having approximately 3 nitric groups per 6 carbon atoms. Most preferred is a Type SMWP material (Millipore Corp., Bedford, Mass.) which has a pore size of 5 μm.

The various chromatographic substrate materials may be used as such in suitable shapes such as films, strips or sheets. They may also be coated onto or bonded or laminated to appropriate inert support materials such as paper, glass, plastic, metal or fabrics. (One preferred inert support material is Mylar.) Such a support material not only has the effect of providing structural support to the chromatographic substrate material but also prevents evaporation of reagent and solvent materials during the assay procedure. Cover plates may also be fashioned of such inert materials. Cover plates, although not required for practice of the invention, lend additional structural support and further prevent evaporation of reagent and solvent materials during the assay procedure. Such cover plates may be transparent for viewing the progression of the assay and may comprise ports for addition of sample materials, chromatographic solvent or reagents.

The chromatographic medium upon which the assays are conducted may be any shape or size but is preferably in the form of strips of thickness in the range of from about 0.01 mm to about 0.5 mm, and most preferably of about 0.1 mm. The strips may vary widely in their other dimensions but are preferably kept fairly small in order to shorten the assay development time and minimize material usage. When the strips are extremely small in size they may be attached to a suitable handle or holder in order to aid in handling and observation of results. Strips approximately 3 mm wide and up to 75 mm long have been found to be particularly suitable in the fabrication of single pathway devices according to the present invention. The pore size may vary within wide limits but is preferably between about 0.05 μm and 20 μm and preferably about 5 μm. Pore size is limited on the lower end by the size of the transported analytes, reagents and colloidal particle labels. If the pore size is too small, assay materials will be transported slowly or not at all. On the higher end, pore size is limited by binding capacity. It is generally desired that chromatographic transport be rapid with the transport and assay being completed within less than five minutes, and preferably less than or about two minutes. Chromatographic transport should not be so rapid that specific binding capacity is lost as reagents do not have time to specifically bind with one another. The combination of pore size and substrate thickness may thus be varied according to the characteristics of the chromatographic solvents, specific reagents, sample materials and colloidal particle labels used in order to obtain desired properties of speed and resolution.

It is desired that in forming the strip materials of the present invention that any irregularities in the materials or in the edges of the materials which might cause uneven flow through the material be avoided. Means of fashioning the strip materials include the use of a paper cutter with a tungsten carbide rotary blade. A preferred means, however, involves the use of laser cutting which is particularly suitable for use in mass production techniques.

Because the chromatographic media of the device is preferably chemically inert, it may have to be activated at any zone where it is desired to immobilize a specific binding reagent against solvent transport. Various methods will be required to render the reagent immobilized according to the particular chemical nature of the substrate material and the second reagent. Generally, when the media is nitrocellulose or a mixed nitrocellulose ester, no special chemical linkage is required for the immobilization of reagents. Various techniques may be used for other materials and reagents which include functionalization with materials such as carbonyldiimidazole, glutaraldehyde or succinic acid, or treatment with materials such as cyanogen bromide. Other suitable reactions include treatment with Schiff bases and borohydride for reduction of aldehydic, carbonyl and amino groups. DNA, RNA and certain antigens may be immobilized against solvent transport by baking onto the chromatographic material. Baking may be carried out at temperatures ranging from about 60° C. to about 120° C. for times varying from about five minutes to about 12 hours, but preferably at about 80° C. for about two hours.

Solvent Transport Barriers

Various means are known for achieving the sequential transport of reagents and sample materials such as are disclosed in co-owned U.S. Pat. No. 4,960,691.

Solvent barriers which block chromatographic flow according to the invention may be formed by various physical or chemical etching techniques. Gaps of less than 0.1 mm in width have been found to prevent the flow of liquid. A preferred means for forming such barriers involves the use of laser etching techniques. A CO_2 laser may be used according to one procedure wherein Mylar backed nitrocellulose is mounted on a supporting fixture which is mounted on a computer controlled X-Y table capable of very close positioning tolerances. Alternatively, a beam moving mechanism may be used. Using a combination of suitable optical lenses and careful beam focusing, a laser beam spot, with a diameter of approximately 0.005 inches, can be focused on the nitrocellulose. By careful control of the laser power, a narrow path of nitrocellulose, approximately 0.005 inches wide, can either be removed from or melted to the Mylar backing. The use of a CO_2 laser is particularly preferred because of the favorable coupling effect of light from the laser with the nitrocellulose. Nevertheless, other types of lasers are suitable, provided that the laser beam wavelength produces the desired effect on the solvent transport material. Through use of a moving beam or an X-Y table, precision paths baffled channels or other intricate shapes may be generated on the nitrocellulose.

Description of the Specific Binding Reagents

Specific binding reagents useful with the present invention include those materials which are members of a specific binding pair consisting of a ligand and a receptor. The ligand and receptor are related in that the receptor specifically binds to the ligand, being capable of distinguishing the ligand from other materials having similar characteristics. The methods, kits and devices according to the present invention are particularly useful in the practice of immunological assay techniques where the specific binding reagents are antigens and antibodies. Specific binding materials such as avidin, biotin, streptavidin and antibiotin may also be labelled with colloidal particles and utilized in chromatographic solvent transport assays according to the invention. The methods, kits and devices may also prove useful in the practice of DNA and RNA hybridization assays and other specific binding assays such as those involving receptors for hormones or other biologically active agents.

Antibodies useful in conducting the immunoassays of the present invention include those specifically reactive with various analytes the detection of which in biological fluids is desired. Such antibodies are preferably IgG or IgM antibodies or mixtures thereof, which are essentially free of association with antibodies capable of binding with non-analyte molecules. The antibodies may be polyclonal or monoclonal and are commercially available or may be obtained by mouse ascites, tissue culture or other techniques known to the art. A typical description of hybridoma procedure for the production of monoclonal antibodies may be found in Wands, J. R., and V. R. Zurawski, Gastroenterology 80:225 (1981); Marshak-Rothstein, A., et al.; J. Immunol. 122:2491 (1979); Oi, V. Y. and L. A. Herzenberg, "Immunoglobulin Producing Hybrid", Mishell, B. B. and S. M. Shiigi (eds.) *Selected Methods in Cellular Immunology*, San Francisco: W. H. Freeman Publishing, 1979; and U.S. Pat. No. 4,515,893 issued to Kung, et al. The use of mixtures of monoclonal antibodies of differing antigenic specificities or of monoclonal antibodies and polyclonal antibodies may be desired. It is further contemplated that fragments of antibody molecules may be used as specific binding reagents according to the invention including half antibody molecules and Fab, Fab' or $F(ab')_2$ fragments known in the art. Regardless of the particular source or type of antibodies, however, it is preferred that they be generally free of impurities. The antibodies may be purified by column chromatographic or other conventional means but are preferably purified according to known affinity purification techniques.

Antigens and haptens useful in carrying out the immunoassays of the present invention include those materials, whether natural or synthesized, which present antigenic determinants for which the analyte antibodies are specifically reactive when presented on the chromatographic strip materials of the invention. Synthesized antigens include those which are constructed according to conventional chemical syntheses as well as those constructed according to recombinant DNA techniques. Antigen materials may also be labelled with enzymes and colloidal particles according to the invention and used in sandwich type assays for the detection of antibody analytes or in competition assays for the detection of antigen analytes.

The methods and devices according to the present invention are expected to be useful in the practice of a wide variety of specific binding assays including nucleic acid hybridization assays. DNA and RNA hybridization materials useful according to the present invention would include DNA and RNA polynucleotide probes having base sequences generally complementary to those of analyte gene materials. The probes of the invention will generally have between about 25 and about 10,000 bases and preferably between about 30 and about 5,000 bases. The probes need not be perfectly complementary to the base sequences of analyte gene materials and will generally hybridize provided about 70% or greater homology exists between the base sequences. Conditions relating to DNA and RNA hybridization are disclosed generally in Crosa, et al., J. Bact. 115(3), 904–911 (1973). Polynucleotide probe materials may be obtained according to techniques well known in the art. See, e.g., Kornberg, DNA Replication, W. H. Freeman and Co., San Francisco, 670–679 (1978); Dallas, et al., J. Bacteriol. 139, 850–858 (1979) and So, et al., Nature, 277, 453–456 (1979).

Description of Blocking Agents

Blocking agents useful in preparation of devices for the specific binding of the present invention are those agents capable of blocking excess binding sites on the chromatographic media which might hinder chromatographic solvent transport of sample materials or reagents of the invention. It is generally not necessary to block the chromatographic substrate material in the practice of mix and run assays where the specific binding reagents are mixed with the sample material and the chromatographic solvent. Blocking of excess binding sites on the chromatographic solvent material is particularly useful, however, where the sample or any reagents are impregnated on the strip in the absence of chromatographic solvent. In the construction of devices of the present invention, the chromatographic media is impregnated with the reagent(s) to be immobilized at the location(s) desired. Once the reagent(s) has (have) been immobilized at the desired zones, the strip is then processed so as to block excess binding sites of the chromatographic material which might interfere with chromatographic solvent transport of other reagents or sample materials. Particularly suitable is the use of blocking solutions comprising proteins from sources such as casein, gelatin or total serum. Such proteins are selected to not interfere with or cross-react with reagent materials of the assays. Blocking of the sites may preferably be conducted by dipping the chromatographic substrate materials in a solution of 0.2% casein in physiological saline and air drying the strip materials. Other methods include dipping in solutions of 0.1% gelatin or 0.1% BSA followed by air drying of the substrate materials.

Description of the Chromatographic Solvent System

Kits for performing "dip and run" assays according to the invention utilize mixtures of the sample materials and indicator solutions themselves for chromatographic transport of the mobile elements of the assays. Where the assay devices are not of the dip and run format and sample materials are applied in smaller quantities to locations not at the first end of the assay devices, chromatographic solvents are required for transport of the various reagents and sample components on the assay devices.

Suitable chromatographic solvent systems for specific binding assays according to the invention are those capable of solubilizing the analyte, labelled reagents and any additional reagents and materials and transporting them on the chromatographic material. Such solvents should have sufficient ionic strength to prevent electrostatic interaction of the transported materials with the strip material. A preferred solvent for use in immunoassay procedures according to the invention is physiological saline solution with a pH in the neutral range. Proteins as well as detergents such as sodium dodecyl sulfate (SDS), polyethylene glycol tert-octyiphenyl ether (TRITON X-100) and sodium deoxycholate (DOC) may be incorporated in the chromatographic solvent in quantities which minimize non-specific binding with the strip material but not in such excess as would prevent the desired binding and immobilization reactions. Other chromatographic solvents such as high performance liquid chromatography (HPLC) solvents and high performance thin layer chromatography (HPTLC) solvents which favor solubilization of proteins and other reactants and minimize binding to strip materials such as nitrocellulose may also be used.

EXAMPLE 1

According to this example, casein was subjected to an alkaline treatment purification procedure. Two hundred grams of essentially vitamin free casein (Sigma Chemical Co., St. Louis, Mo., catalogue no. C-3400) was mixed with 800 ml of distilled water. One liter of 2 M sodium hydroxide was then added, followed by 4 ml of 30% hydrogen peroxide and the mixture was mixed overnight at room temperature.

The material was filtered through Whatman No. 1 filter paper on a Buchner funnel and approximately 94.6 ml of 100% (glacial) acetic acid was added to the filtrate to bring the pH to 7.5. The mixture was again filtered, as before, and approximately 220 ml of acetic acid was added to the filtrate to bring the pH to about 4.5. The mixture was incubated for 30 minutes during which time a large taffy-like lump fell out of solution. The supernate was centrifuged at 2800 rpm in a small RC5C centrifuge for 30 minutes and the pellet was added to the taffy-like lump which was then washed with deionized water.

The taffy-like material was then stirred and dissolved in one liter of 0.15 M aqueous ammonia solution. (In the event that the casein does not go into solution, concentrated (15 M) ammonium hydroxide solution should be added until the pH reaches 7.5.) The casein was then lyophilized overnight when it was completely dried, having a yield of 136 grams.

EXAMPLE 2

According to this example, a colloidal gold/antibody conjugate was produced for practice of the methods of the present invention. Siliconized glassware (Sigma silicote) was utilized throughout the procedure wherein 200 ml of 0.01% gold chloride ($HAuCl_4.3H_2O$) (Fisher Scientific, G-54-1) was brought to a boil and 2 ml of 1% sodium citrate solution was added and the boiling is continued for 5 minutes until the color of the solution changes from pale yellow to purple to red. A solution of potassium carbonate

(0.02M) was added to the suspension in order to adjust the pH to 7.6, followed by addition of goat anti-human IgG (1 mg/ml) (Kirkegaard-Perry, Gaithersburg, Md.) such that approximately 10 μg IgG was added per ml of gold suspension (0.01% gold).

After one minute of incubation at room temperature, 0.1 ml of a solution of 30% bovine serum albumin in water was added to 10 ml of the gold suspension. Aggregated material was removed by centrifugation at 3000 rpm in the SS34 rotor of a Sorval RC5C centrifuge for 10 minutes. The supernate was subjected to an additional centrifugation step at 6000 rpm for one hour. The colloidal gold conjugate in the pellet was resuspended in 2% bovine serum albumin in PBS (0.05 M potassium phosphate buffer, pH 7.4, in 0.9% NaCl), the preferred conditions for liquid storage being at 4° C. The meta-soluble preparation of casein, prepared as in Example 1, was added to give a concentration of 1% immediately before application to the membrane. The conjugate was then stored for prolonged periods in the dry state, with no loss of activity after 6 months storage at 37° C. in the dry state.

EXAMPLE 3

According to this example, sandwich-type immunoassay devices for the detection of rubella antibodies were constructed and used. Microporous nitrocellulose material with a thickness of approximately 0.1 mm and an average pore size of 5 μm was laminated with mylar and adhesive (Monokote, Top Flite Models, Inc., Chicago, Ill.). Strips measuring 1 cm by 3.5 cm were cut by high powered laser and solvent transport lanes and a delaying box were fashioned by laser etching according to the general design of the device of FIG. 4. Rubella antigen (Abbott Laboratories, North Chicago, Ill.) (0.2 μl, 2500 HA titer) was applied to the strips at a third zone where it was immobilized and air dried. Non-specific binding sites on the chromatographic strip materials were then blocked by incubation for 10 minutes at room temperature with a 0.1% solution of LB gelatin in water (Inotech, Wohlen, Switzerland) and the strips were allowed to dry under a stream of air. One μl of gold particle labelled goat anti-human IgG in an anti-aggregation buffer produced according to Example 2 was then applied to a first zone of each strip (adjacent to the delay box) and dried.

Positive and negative serum samples for the rubella antibody were then applied to the second zones between the first and third zones and the first end of the strips were dipped into a chromatographic transport solvent comprising TBS and 1% TRITON X-100 (polyethylene glycol tert-octylphenyl ether). The liquid front was allowed to progress to the second ends of the devices over a period of approximately 2.5 minutes transporting the sample material and the gold labelled goat anti-human IgG to the third zone. Positive sera and the immobilization of the labelled first reagent resulted in the presence of a red spot at the third zone. Strips tested with negative sera did not produce a signal at the third zone.

EXAMPLE 4

In this example a mix and run sandwich-type immunoassay device for the detection of human chorionic gonadotropin (HCG) was constructed and used. The device which is fashioned of the same general design as the device of FIG. 2 produces a signal confirming the presence of a labelled first specific reagent in the sample as a negative control and produces an additional signal indicating the presence of the HCG analyte. Microporous nitrocellulose material with a thickness of approximately 0.1 mm and an average pore size of 5 μm was laminated with mylar and cement (Monokote) according to the methods of Example 3.

At a first zone, the strips were impregnated with a second reagent comprising 0.35 μl of 2 mg/ml anti-HCG polyclonal antibody in buffered saline containing 1% sucrose. At a second zone the strips were impregnated with a third reagent comprising 0.45 μl of a 100 ug/ml goat anti-mouse IgG in Tris buffered saline containing 1% sucrose. The two zones were located approximately 10 mm from the first end of the strip and were arranged such that the second zone was in the form of a minus (−) sign and the first zone was located on two sides of the second zone so that the two zones together form a plus (+) sign.

Anti-HCG antibodies (Abbott Laboratories, North Chicago, Ill.) were incubated with 1 ml of colloidal gold suspension adjusted to pH 6.6 with potassium carbonate according to the method of Example 2 such that approximately 10 μg IgG was added per ml of gold suspension. Indicator solution was then prepared comprising 10 μl of colloidal gold labelled anti-HCG antibody. The gold particle labelled antibodies were added to 10 μl of Tris-buffered saline containing 10% alkaline treated casein according to Example 1 and 1% PEG (M.W. 20,000). The indicator solution was then mixed with 100 μl of a urine sample to which varying amounts of HCG had been added.

The test strip was then contacted at its first end in the mixture of sample and indicator solution and the liquid front was allowed to rise through the zones to the second end of the strip. When the sample solution did not contain any of the HCG antigen the mixture of sample and indicator solution progressed through the strip. Upon contacting the second zone where the goat anti-mouse IgG had been immobilized the labelled anti-HCG antibodies were immobilized by the selective immunological reaction. As no HCG was present in the sample solution there was no specific binding with the second reagent immobilized at the first zone. The colloidal gold labelled reagents thus produced a visually detectable signal in the form of a minus (−) sign indicating operability of the reagents but absence of HCG in the sample.

When the sample solution contained HCG the labelled anti-HCG antibodies selectively bound to the analyte to form a labelled conjugate. The mixture of the sample solution and the indicator solution was transported by chromatographic solvent transport through the first and second zones to the second end. Upon contacting the first zone where polyclonal anti-HCG antibodies had been immobilized the gold labelled antibody/HCG conjugate was immobilized by a specific binding reaction of the HCG antigen with the anti-HCG antibodies. At the same time, the HCG/antibody conjugates and any unconjugated labelled anti-HCG antibodies contacting the second zone were immobilized by contacting the goat anti-mouse IgG antibodies immobilized at that zone. The colloidal gold labelled reagents thus immobilized at both the first and second zones produced a visually detectable signal in the form of a plus (+) sign indicating the presence of HCG in the sample. The sensitivity for HCG of this format was determined to be as low as 25 milli-IU/ml.

EXAMPLE 5

In this example, a mix and run sandwich-type immunoassay for the detection of A-polysaccharide (APS) was prepared and used according to the methods of Example 4. According to this example, nitrocellulose strips were pre-

pared according to Example 4 and were treated with polyclonal anti-APS antibodies which were immobilized at the first zone.

Rabbit polyclonal anti-APS antibodies (Abbott Laboratories, North Chicago, Ill.) were incubated with 1 ml of colloidal gold suspension adjusted to pH 7.2 with potassium carbonate according to the method of Example 2 such that approximately 10 μg of anti-APS antibody was added per ml of gold suspension. An indicator solution was then prepared comprising 10 μl of colloidal gold labelled anti-APS antibodies. The gold particle labelled antibodies were added to 10 μl of Tris-buffered saline containing 10% alkaline treated casein according to Example 1 and 1% PEG. The indicator solution was then mixed with 100 μl of a swab extraction buffer for strep to which varying amounts of APS (Abbott Laboratories, North Chicago, Ill.) had been added.

The test strip was then dipped at its first end in the mixture of a sample and indicator solution and the liquid front was allowed to rise through the first zone to the second end of the strip. APS present in the samples reacted with and was bound to the anti-APS antibodies in the indicator solution to form a conjugate. These conjugates were then immobilized at the first zone by reaction between the APS and the anti-APS polyclonal antibodies immobilized at the zone. The presence of APS in the swab extracted sample was indicated by the development of a purple color as a consequence of the concentration of the colloidal gold particles at the zone. The sensitivity for APS of devices according to this format was determined to be as low as 0.5 ng/ml APS.

EXAMPLE 6

In this example, sandwich-type immunoassay devices for the detection of swine anti-trichina antibodies were produced according to the general procedures of Example 3. Nitrocellulose assay strips were prepared and were treated with partially purified trichina antigen (United States Department of Agriculture) immobilized at a detection zone.

Colloidal selenium particles of various sizes were produced according to the methods of co-owned U.S. Pat. No. 4,954,452. Various volumes (40, 80 and 150 μl aliquots) of concentrated selenium sol were pipetted into individual vials containing 4 ml of water each and the pH of each solution was adjusted to 7.2 by addition of 0.01 M potassium carbonate. To each of the vials was then added 150 μl of goat anti-swine antibody (1 mg/ml concentration) (Kirkegaard-Perry). The solutions were mixed and allowed to incubate for 10 minutes. A 0.5 ml aliquot of a 0.5% solution of alkaline treated casein was added to each solution and mixed well. Three ml aliquots of each selenium conjugate solution were centrifuged in 1 ml portions on a TDx table centrifuge and the pellets were combined for each conjugate after the supernatant was decanted off. The combined pellets of each conjugate were resuspended with a solution of 4% casein in 20 μl of TBS. 0.5 μl aliquots of the selenium particle labelled antibody indicator solutions were then applied to a first zone of each strip (adjacent to the delay box) and dried.

Positive and negative serum samples containing Trichina antibodies were then applied to the second zones of the devices between the first and third zones and the first end of the strips were dipped into a chromatographic transport solvent comprising TBS and 1% TRITON x-100 (polyethylene glycol tert-octylphenyl ether). The liquid front was allowed to progress to the second ends of the devices over a period of approximately 2.5 minutes, transporting the sample material and the selenium labelled anti-swine antibodies to the third zone. All conjugates gave visible positive signals with the conjugate utilizing 80 nm selenium particles providing the best results. All the conjugate solutions were tested against a negative control which indicated no specific binding.

EXAMPLE 7

In this example, a mix and run sandwich-type immunoassay device was constructed and used according to the general procedures of Example 4. Instead of incubating the anti-HCG antibodies with gold particles the antibodies were incubated with colloidal selenium particles produced according to the methods of co-owned U.S. Pat. No. 4,954,452 Selenium particles of varying sizes were tested against varying concentrations of HCG. The conjugate utilizing 80 nm particles gave the best results with a detection limit of 20 mIU/ml. Antibodies labelled with larger or smaller particles gave less sensitive results as shown in Table 1 below. All conjugate solutions were tested against a negative control which indicated no specific binding.

TABLE 1

Particle Size (nm)	Detection Limit (mIU/ml)
11	500
80	20
140	50
193	50
300	4000

EXAMPLE 8

According to this example, sandwich-type immunoassay devices for the detection of rubella antibodies were constructed and used according to the general procedures of Example 3. Instead of the gold particle labelled goat anti-human IgG, however, alkaline phosphatase labelled goat anti-human IgG (Kirkegaard-Perry) was used to detect the presence of rubella antibodies immobilized at the second zones. One μl of 1 mg/ml of the alkaline phosphatase labelled IgG was diluted in a PBS solution containing 1% alkaline treated casein and was applied to the first zone of each strip and dried. The assay devices could then be constructed, stored for prolonged periods and used in the manner of the gold labelled assay devices of Example 3 with the exception that enzyme substrate and indicator dye reagents must be added to the test strips in order to visualize the assay results.

Numerous modifications and variations in practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing descriptions of preferred embodiments thereof. The use of colloidal particle labelled reagents in chromatographic assay techniques is of wide applicability and is not limited to the specific examples disclosed. It is thus, well within the skill in the art to practice the present invention according to a wide variety of methods and formats. Consequently, only such limitations should be placed on the invention as appear in the following claims.

What is claimed is:

1. A method for determining the presence or amount an analyte in a test sample, the method comprising the steps:

a) contacting a chromatographic medium with a test sample, wherein the medium comprises at least two reaction sites;

a first reaction site comprising a dried colloidal particle labeled specific binding reagent and a chromatographic transport facilitating agent, and

a second reaction site comprising an immobilized specific binding agent capable of immobilizing said colloidal particle labeled specific binding reagent;

b) solubilizing said colloidal particle labeled specific binding reagent and said chromatographic transport facilitating agent and chromatographic ally transporting the test sample and at least a portion of said colloidal particle labeled specific binding reagent from the first reaction site to the second reaction site by capillary interaction of said test sample and said chromatographic medium; and

c) detecting said colloidal particle specific binding reagent at said second reaction site to determine the presence or amount of the analyte in the test sample.

2. The method according to claim 1 wherein said chromatographic transport facilitating agent is a meta-soluble protein.

3. The method according to claim 1 wherein said chromatographic transport facilitating agent is casein.

4. The method according to claim 1 wherein said chromatographic transport facilitating agent is polyethylene glycol.

5. The method according to claim 1 wherein said chromatographic transport facilitating agent is gelatin.

6. The method according to claim 1 wherein said chromatographic transport facilitating agent is bovine serum albumin.

7. The method according to claim 1 wherein said chromatographic transport facilitating agent is a detergent.

8. The method according to claim 1 wherein said colloidal particles have a diameter ranging from about 1 nm to about 200 nm.

9. The method according to claim 8 wherein said colloidal particles have a diameter ranging from about 40 nm to about 80 nm.

10. The method according to claim 1 wherein said colloidal particles are colloidal metal particles.

11. The method according to claim 10 wherein said colloidal metal particles are colloidal gold particles.

12. The method according to claim 1 wherein said colloidal particles are non-metal colloidal particles.

13. The method according to claim 12 wherein said colloidal particles are colloidal selenium particles.

* * * * *

AO 88 (Rev. 1/94) Subpoena in a Civil Case

Issued by the

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

ABBOTT LABORATORIES et al. Plaintiffs V. CHURCH & DWIGHT CO., INC. Defendants	**SUBPOENA IN A CIVIL CASE** CASE NUMBER:[1] 07-CV-3428 (MFK) (For a case pending in the Northern District of Illinois)

TO:
Scantibodies Laboratory, Inc.
ATTN: ALLEN GARRETT, Registered Agent
9336 Abraham Way
Santee, CA 92071 USA

YOU ARE COMMANDED to appear in the United States District Court at the place, date, and time specified below to testify in the above case.

PLACE OF TESTIMONY	COURTROOM
	DATE AND TIME

☒ YOU ARE COMMANDED to appear at the place, date, and time specified below to testify at the taking of a deposition in the above case.

PLACE OF DEPOSITION	DATE AND TIME
See the attached notice of deposition and Exhibit A, pursuant to Federal Rule of Civil Procedure 30(b)(6)	May 23, 2008 9:00 a.m.

☒ YOU ARE COMMANDED to produce and permit inspection and copying of the following documents or objects at the place, date, and time specified below (list documents or objects):

See Schedule B, attached

PLACE	DATE AND TIME
Merrill Corporation British Pacific Centre 8899 University Center Lane, Suite 200 San Diego, CA 92122	May 12, 2008 10:00 a.m.

YOU ARE COMMANDED to permit inspection of the following premises at the date and time specified below.

PREMISES	DATE AND TIME

Any organization not a party to this suit that is subpoenaed for the taking of a deposition shall designate one or more officers, directors, or managing agents, or other persons who consent to testify on its behalf, and may set forth, for each person designated, the matters on which the person will testify. Federal Rules of Civil Procedure, 30(b)(6).

ISSUING OFFICER SIGNATURE AND TITLE (INDICATE IF ATTORNEY FOR PLAINTIFF OR DEFENDANT)	DATE
Stephanie S. McCallum, Esq., Attorney for Defendant Abbott Laboratories	April 25, 2008

ISSUING OFFICER'S NAME, ADDRESS AND PHONE NUMBER
Stephanie S. McCallum., Esq., Winston & Strawn LLP, 35 W. Wacker Dr., Chicago, IL 60601 (312) 558-5600

(See Rule 45, Federal Rules of Civil Procedure, Parts C & D on Reverse)

[1] If action is pending in district other than district of issuance, state district under case number.

AO 88 (Rev. 1/94) Subpoena in a Civil Case

PROOF OF SERVICE

DATE PLACE

SERVED

SERVED ON (PRINT NAME) MANNER OF SERVICE

SERVED BY (PRINT NAME) TITLE

DECLARATION OF SERVER

I declare under penalty of perjury under the laws of the United States of America that the foregoing information contained in the Proof of Service is true and correct.

Executed on _____

DATE SIGNATURE OF SERVER

ADDRESS OF SERVER

Rule 45, Federal Rules of Civil Procedure, Parts C & D:

(c) PROTECTION OF PERSONS SUBJECT TO SUBPOENAS.

(1) A party or an attorney responsible for the issuance and service of a subpoena shall take reasonable steps to avoid imposing undue burden or expense on a person subject to that subpoena. The court on behalf of which the subpoena was issued shall enforce this duty and impose upon the party or attorney in breach of this duty an appropriate sanction which may include, but is not limited to, lost earnings and reasonable attorney's fee.

(2) (A) A person commanded to produce and permit inspection and copying of designated books, papers, documents or tangible things, or inspection of premises need not appear in person at the place of production or inspection unless commanded to appear for deposition, hearing or trial.

(B) Subject to paragraph (d) (2) of this rule, a person commanded to produce and permit inspection and copying may, within 14 days after service of subpoena or before the time specified for compliance if such time is less than 14 days after service, serve upon the party or attorney designated in the subpoena written objection to inspection or copying of any or all of the designated materials or of the premises. If objection is made, the party serving the subpoena shall not be entitled to inspect and copy materials or inspect the premises except pursuant to an order of the court by which the subpoena was issued. If objection has been made, the party serving the subpoena may, upon notice to the person commanded to produce, move at any time for an order to compel the production. Such an order to compel production shall protect any person who is not a party or an officer of a party from significant expense resulting from the inspection and copying commanded.

(3) (A) On timely motion, the court by which a subpoena was issued shall quash or modify the subpoena if it

(i) fails to allow reasonable time for compliance;

(ii) requires a person who is not a party or an officer of a party to travel to a place more than 100 miles from the place where that person resides, is employed or regularly transacts business in person, except that, subject to the provisions of clause (c) (3) (B) (iii) of this rule, such a person may in order to attend trial be commanded to travel from any such place within the state in which the trial is held, or

(iii) requires disclosure of privileged or other protected matter and no exception or waiver applies, or

(iv) subjects a person to undue burden.

(B) If a subpoena

(i) requires disclosure of a trade secret or other confidential research, development, or commercial information, or

(ii) requires disclosure of an unretained expert's opinion or information not describing specific events or occurrences in dispute and resulting from the expert's study made not at the request of any party, or

(iii) requires a person who is not a party or an officer of a party to incur substantial expense to travel more than 100 miles to attend trial, the court may, to protect a person subject to or affected by the subpoena, quash or modify the subpoena, or, if the party in whose behalf the subpoena is issued shows a substantial need for the testimony or material that cannot be otherwise met without undue hardship and assures that the person to whom the subpoena is addressed will be reasonably compensated, the court may order appearance or production only upon specified conditions.

(d) DUTIES IN RESPONDING TO SUBPOENA.

(1) A person responding to a subpoena to produce documents shall produce them as they are kept in the usual course of business or shall organize and label them to correspond with the categories in the demand.

(2) When information subject to a subpoena is withheld on a claim that it is privileged or subject to protection as trial preparation materials, the claim shall be made expressly and shall be supported by a description of the nature of the documents, communications, or things not produced that is sufficient to enable the demanding party to contest the claim.

EXHIBIT A

I. DEFINITIONS

1. "Scantibodies" or "you" or "your" shall mean Scantibodies Laboratory, Inc., as well as its predecessors, successors, subsidiaries, parent companies, agents, representatives, partners, employees, affiliates or persons purporting to act on its behalf, including, but not limited to, Church & Dwight Co., Inc., Carter-Wallace, Inc. and Armkel LLC.

2. "C&D" or "Defendant" shall mean Church & Dwight, Co., Inc., the Defendant and Counterclaimant in this action, as well as its predecessors, successors, subsidiaries, parent companies, agents, representatives, partners, employees, affiliates or persons purporting to act on its behalf, including, but not limited to, Carter-Wallace, Inc. and Armkel LLC.

3. "Plaintiff" or "Abbott" shall mean Abbott Laboratories, Plaintiff in this action, as well as its predecessors, successors, subsidiaries, parent companies, agents, representatives, partners, employees, affiliates or persons purporting to act on its behalf.

4. "C&D Test Kit" shall mean any diagnostic product that is used to test for the presence and/or quantity of a ligand and was manufactured in whole or in part by Scantibodies on behalf of C&D and sold or otherwise delivered to C&D for resale, including but not limited to, any pregnancy and ovulation tests manufactured, made, used or sold by or on behalf of C&D from 1999 to the present such as the pregnancy and ovulation predictor tests sold to consumers with the label First Response, Answer or Answer Quick & Simple.

5. "Document" shall mean any written, graphic, recorded or illustrative material of any kind or description, however produced or reproduced, and regardless of whether approved, signed, sent, received, redrafted, or executed, prepared by or for you, in your possession, custody, or control. The term "document" includes, but is not limited to, the following: correspondence, memoranda, drafts, computerized records, notes, jottings, books, lab notebooks,

records, reports, surveys, studies, analyses, things, videotapes, recordings, computer disks, electronic mail, e-mail, transcriptions of verbal conversations or statements however made, business forms, labels, papers and films filed with courts or other governmental bodies, notices, messages, calendar and diary entries, appointment books, minutes and other formal or informal memoranda of meetings, and copies of documents that are not identical duplicates of the originals (*e.g.*, because handwritten or "blind" notes appear thereon or are attached thereto).

6. "Thing" shall mean any physical specimen or tangible item other than a document.

7. "Person" shall mean any natural person, firm, association, partnership, government agency, or other entity and its officers, directors, partners, employees, representatives and agents.

8. The term "relating to" "reflecting," "referring," "relating to," or any derivation thereof shall mean, without limitation, consisting of, constituting, containing, mentioning, describing, summarizing, evidencing, listing, indicating, analyzing, explaining, supporting, undermining, contradicting, concerning, reflecting, referring to, pertaining to, prepared in connection with, used in preparation for, or being in any way legally, logically, or factually connected with the matter discussed.

9. "Communication" shall mean or refer to all inquires, discussions, conversations, negotiations, agreements, understandings, meetings, telephone conversations, letters, notes, telegrams, advertisements, or other forms of information exchanged, whether oral, electronic, or written. Unless the request specifically states otherwise, references to the singular shall include the plural and vice versa; references to one gender shall include the other gender; references to the past include the present and vice versa; and disjunctive terms include the conjunctive and vice versa.

10. If you at any time had possession or control of a document requested herein and if such document has been lost, destroyed, purged, or is not presently in your possession or control, identify the document, the date of its loss, destruction, purge or separation from your possession or control, and the circumstances surrounding its loss, destruction, purge or separation from your possession or control.

11. Should you refuse, on the grounds of attorney-client privilege, work product immunity or any other privilege, to produce or testify about any document or tangible thing, then you shall provide at the time of making said refusal a list of all such non-produced documents or things. As to any such document or thing, state the following: the nature of the privilege or immunity being claimed; the number of the request calling for its production; the date of the document; the name of each person who signed or prepared the document; the name of each addressee and person to whom the document or copies thereof were given or sent; a description of the general subject matter of the document; an identification of any document or other material transmitted with or attached to the document; and the nature or character of the document or thing.

12. The "SurModics Patents" shall mean U.S. Patent Nos. 5,654,162, and 6,020,147.

13. The "'162 patent" shall mean U.S. Patent No. 5,654,162.

14. The "'147 patent" shall mean U.S. Patent No. 6,020,147.

15. The term "Patents-in-Suit" shall mean the SurModics Patents.

16. The "Asserted Claims" shall mean claims 1, 7, 9, 10, 16, 17, 19, 20, 22, 28, and 29 of the '162 patent and claims 1, 5, 6, 7, 11 and 12 of the '147 patent. Discovery still is in its early stages and Abbott reserves the right to assert additional claims as it receives additional information and discovery about C&D's products.

II. DEPOSITION TOPICS

1. The manufacture, location of manufacture, structure, method of operation, components, ingredients and materials of C&D Test Kit, including but not limited to, the antibodies used in each such test; the release, resuspension, or dispersion of labeled antibodies when contacted by a liquid sample; the amount of such antibodies and reagents used to manufacture the C&D Test Kits; the binding of an analyte to a labeled reagent; all immunoassay reactions, chemical reactions, antibody reactions, and analyte reactions; the application, immobilization and mobilization of antibodies on or in a test strip; the detection of a test result; and any and all internal controls for quality, function, operation or detection.

2. The use, composition, function and properties (including binding properties) of biotin, avidin, streptavidin and/or NeutrAvidin in each C&D Test Kit, and all tests, analyses and evaluations concerning the biotin, avidin, streptavidin and/or NeutrAvidin used in the test kit.

3. The use, composition, function and properties (including binding properties) of antibodies in each C&D Test Kit, including but not limited to antibodies designated as 2B2, B109, LH 26, 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 5304, and all tests, analyses and evaluations concerning any or all of those antibodies.

4. The research, design, development, and testing of each C&D Test Kit.

5. The identification of documents sufficient to describe the manufacture, location of manufacture, structure, method of operation, components, ingredients, nature and property of each ingredient or component used, and materials used in the manufacture and production of each C&D Test Kit. The authenticity and business record status of any such document identified.

6. Identification of documents and samples sufficient to show each model and/or version of each C&D Test Kit as used by Scantibodies. The authenticity and business record status of any document or sample identified.

7. The circumstances by which Scantibodies became aware of the Patents-in-Suit or any related patent, patent application, or foreign counterparts, and all communications by or between Scantibodies and C&D, and/or any other third party, regarding the Patents-in-Suit, including for each patent or application, the earliest date by which Scantibodies became aware of the patent or application, any actions that Scantibodies took or that others took on behalf of Scantibodies after Scantibodies first became aware of the patent or application; and the identity of all persons who became aware of the patent or application, or took actions by or on behalf of Scantibodies after Scantibodies became aware of the patent or application, including but not limited to what actions were taken.

8. All communications with C&D regarding, relating to and/or concerning the Patents in Suit.

9. All attempts, efforts or actions that C&D, Scantibodies, and/or others took on its behalf to design around the claims of the Patents-in-Suit.

10. All opinions of counsel (oral or written) requested and/or received by Scantibodies relating to the Patents-in-Suit, including who provided the opinions, when the opinions were received, to whom the opinions were given, the substance of the opinions, and any actions C&D took as a result of those opinions.

11. Scantibodies's knowledge of, possession of, study of, testing of, and other activities related to any test kit sold by Abbott Laboratories used to diagnose pregnancy.

12. For C&D Test Kits manufactured by Scantibodies in whole or in part and sold or otherwise delivered to C&D from 1999 to the present, the number of units sold on a monthly and

annual basis, total sales figures, including gross sales income on a monthly and annual basis, the costs associated with the sales, including any amounts paid as royalties or licensing fees, the amount of profits attributable to the sales, and how profit related to those sales is calculated.

13. The identification of documents sufficient to show the information requested in Request No. 12 and the authenticity and business record status of such documents.

14. Any agreement between C&D and Scantibodies regarding indemnification in connection with third party patent for the manufacture and/or sale of the C&D test Kits, including but not limited to communications between C&D and Scantibodies concerning any such agreement.

SCHEDULE B

Definitions and Instructions

1. "Scantibodies" or "you" or "your" shall mean Scantibodies Laboratory, Inc., as well as its predecessors, successors, subsidiaries, parent companies, agents, representatives, partners, employees, affiliates or persons purporting to act on its behalf, including, but not limited to, Church & Dwight Co., Inc., Carter-Wallace, Inc. and Armkel LLC.

2. "C&D" or "Defendant" shall mean Church & Dwight, Co., Inc., the Defendant and Counterclaimant in this action, as well as its predecessors, successors, subsidiaries, parent companies, agents, representatives, partners, employees, affiliates or persons purporting to act on its behalf, including, but not limited to, Carter-Wallace, Inc. and Armkel LLC.

3. "Plaintiff" or "Abbott" shall mean Abbott Laboratories, Plaintiff in this action, as well as its predecessors, successors, subsidiaries, parent companies, agents, representatives, partners, employees, affiliates or persons purporting to act on its behalf.

4. "C&D Test Kit" shall mean any diagnostic product that is used to test for the presence and/or quantity of a ligand and was manufactured in whole or in part by Scantibodies on behalf of C&D and sold or otherwise delivered to C&D for resale, including but not limited to, any pregnancy and ovulation tests manufactured, made, used or sold by or on behalf of C&D from 1999 to the present such as the pregnancy and ovulation predictor tests sold with the label First Response, Answer or Answer Quick & Simple.

5. "Document" shall mean any written, graphic, recorded or illustrative material of any kind or description, however produced or reproduced, and regardless of whether approved, signed, sent, received, redrafted, or executed, prepared by or for you, in your possession, custody, or control. The term "document" includes, but is not limited to, the following:

correspondence, memoranda, drafts, computerized records, notes, jottings, books, lab notebooks, records, reports, surveys, studies, analyses, things, videotapes, recordings, computer disks, electronic mail, e-mail, transcriptions of verbal conversations or statements however made, business forms, labels, papers and films filed with courts or other governmental bodies, notices, messages, calendar and diary entries, appointment books, minutes and other formal or informal memoranda of meetings, and copies of documents that are not identical duplicates of the originals (*e.g.*, because handwritten or "blind" notes appear thereon or are attached thereto).

6. "Thing" shall mean any physical specimen or tangible item other than a document.

7. "Person" shall mean any natural person, firm, association, partnership, government agency, or other entity and its officers, directors, partners, employees, representatives and agents.

8. The term "relating to" "reflecting," "referring," "relating to," or any derivation thereof shall mean, without limitation, consisting of, constituting, containing, mentioning, describing, summarizing, evidencing, listing, indicating, analyzing, explaining, supporting, undermining, contradicting, concerning, reflecting, referring to, pertaining to, prepared in connection with, used in preparation for, or being in any way legally, logically, or factually connected with the matter discussed.

9. "Communication" shall mean or refer to all inquires, discussions, conversations, negotiations, agreements, understandings, meetings, telephone conversations, letters, notes, telegrams, advertisements, or other forms of information exchanged, whether oral, electronic, or written. Unless the request specifically states otherwise, references to the singular shall include the plural and vice versa; references to one gender shall include the other gender; references to the past include the present and vice versa; and disjunctive terms include the conjunctive and vice versa.

10. If you at any time had possession or control of a document requested herein and if such document has been lost, destroyed, purged, or is not presently in your possession or control, identify the document, the date of its loss, destruction, purge or separation from your possession or control, and the circumstances surrounding its loss, destruction, purge or separation from your possession or control.

11. Should you refuse, on the grounds of attorney-client privilege, work product immunity or any other privilege, to produce any document or tangible thing, then you shall provide at the time of making said refusal a list of all such non-produced documents or things. As to any such document or thing, state the following: the nature of the privilege or immunity being claimed; the number of the request calling for its production; the date of the document; the name of each person who signed or prepared the document; the name of each addressee and person to whom the document or copies thereof were given or sent; a description of the general subject matter of the document; an identification of any document or other material transmitted with or attached to the document; and the nature or character of the document or thing.

12. The "SurModics Patents" shall mean U.S. Patent Nos. 5,654,162, and 6,020,147.

13. The "'162 patent" shall mean U.S. Patent No. 5,654,162.

14. The "'147 patent" shall mean U.S. Patent No. 6,020,147.

15. The term "Patents-in-Suit" shall mean the SurModics Patents.

16. The "Asserted Claims" shall mean claims 1, 7, 9, 10, 16, 17, 19, 20, 22, 28, and 29 of the '162 patent and claims 1, 5, 6, 7, 11 and 12 of the '147 patent. Discovery still is in its early stages and Abbott reserves the right to assert additional claims as it receives additional information and discovery about C&D's products.

List of Documents

1. All documents and things relating to any agreements between Scantibodies and C&D and/or any other party relating to the manufacture of any C&D Test Kit, including but not limited to any such agreements themselves.

2. All documents and things relating to any obligation by Scantibodies to indemnify or otherwise reimburse or contribute to payment by C&D of any damages awarded to Abbott as a result of C&D's alleged infringement of the Patents-In-Suit.

3. All documents and things relating to any obligation by C&D to indemnify or otherwise reimburse or contribute to payment by Scantibodies of any damages awarded to Abbott as a result of C&D's alleged infringement of the Patents-In-Suit.

4. All communications between Scantibodies and C&D relating to the decision to enter into, or to forgo, any agreement relating to the manufacture of any C&D Test Kit.

5. All communications between Scantibodies and C&D relating to the manufacture of any C&D Test Kit.

6. All documents relating to materials, ingredients or components used in the manufacture of any C&D Test Kit.

7. All documents relating to the manufacture by Scantibodies of any C&D Test Kit, including manufacturing processes, manufacturing specifications, raw materials specifications, and engineering drawings.

8. All documents relating to the design and development of any C&D Test Kit.

9. All documents and things relating to and/or referring to any antibody used in the C&D Test Kit including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

10. Samples of each of the antibodies used in the C&D test kits from 1999 to the present, including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

11. All tests, analyses, evaluations and/or documents referring to, relating to and/or performed with each of the antibodies used in the C&D Test Kits, including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

12. All documents and things relating to and/or referring to the binding capabilities of each of the antibodies used in the C&D Test Kits, including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

13. Samples of the streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

14. All documents and things relating to and/or referring to the streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

15. All tests, analysis, evaluations and/or documents referring to, relating to and/or performed with the streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

16. All documents and things relating to and/or referring to the binding capabilities of the streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

17. All documents and things relating to the amount of each of the antibodies used in the C&D Test Kits, including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

18. All documents and things relating to the amount of streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

Edward Patrick Swan, Jr., State Bar No. 089429
LUCE, FORWARD, HAMILTON & SCRIPPS LLP
600 West Broadway, Suite 2600
San Diego, California 92101-3372
Telephone No.: 619.699.2415
Fax No.: 619.645.5321

Attorneys for Non-Party Scantibodies Laboratory, Inc.

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

ABBOTT LABORATORIES,

Plaintiff,

v.

CHURCH & DWIGHT CO., INC.,

Defendant.

Case No. 07-CV-3428 (MFK)
(Pending in the Northern District of Illinois)

NON-PARTY SCANTIBODIES LABORATORY, INC.'S RESPONSES AND OBJECTIONS TO PLAINTIFF ABBOTT LABORATORIES' SUBPOENA DUCES TECUM

Non-party Scantibodies Laboratory, Inc. ("Scantibodies") hereby responds and objects to plaintiff Abbott Laboratories' ("Abbott") subpoena duces tecum dated April 25, 2008 (the "Subpoena") as follows:

GENERAL OBJECTIONS

1. Scantibodies objects that the Subpoena seeks a broad range of documents that Scantibodies believes is largely in the possession of defendant Church & Dwight Co., Inc. and is the subject of pending discovery requests by Abbott of Church & Dwight Co., Inc. Abbott is required to seek the documents first from Church & Dwight Co., Inc., and only pursue them from Scantibodies, a non-party, if it has exhausted all attempts to secure them from Church & Dwight Co., Inc. Accordingly, the Subpoena is premature and objectionable.

2. Scantibodies objects to the Subpoena, including the "Definitions and Instructions" set forth in Schedule B thereto, to the extent it purports to impose burdens, obligations or

requirements on Scantibodies that are inconsistent with, or exceed the scope of, permissible discovery under the Federal Rules of Civil Procedure.

3. Scantibodies objects to the "Definitions and Instructions" set forth in Schedule B to the extent they collectively fail to describe the information requested with reasonable particularity and attempt to impose obligations on Scantibodies that are not required by, or consistent with, the Federal Rules of Civil Procedure.

4. Scantibodies objects to the "List of Documents" set forth in Schedule B (the "Requests") to the extent they seek the production of documents that disclose any information, materials or communications protected from disclosure by the attorney-client privilege or any other applicable privileges.

5. Scantibodies objects to the Requests to the extent they seek the production of documents containing confidential or proprietary business information.

6. Scantibodies objects to the Requests to the extent they seek the production of documents that are not within its possession, custody or control.

7. Scantibodies objects to the Requests to the extent they seek the production of documents that are already in the possession, custody or control of Abbott, or that are as equally accessible to Abbott as they are to Scantibodies.

8. Scantibodies objects to the Requests to the extent they seek the production of documents that are not relevant or reasonably calculated to lead to the discovery of admissible evidence.

9. Scantibodies objects to the Requests to the extent they are unlimited in scope and/or time, or pertain to a period of time that is not relevant to the underlying action.

10. Scantibodies objects to the Requests to the extent they seek documents that were not prepared, reviewed, sent or received by Scantibodies, and that have no connection to Scantibodies or its business.

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11. Scantibodies' responses are submitted without waiving, and while specifically preserving: (a) all objections as to the competency, relevancy, materiality and admissibility of the responses or the documents produced; (b) all objections to any demand for additional production of documents; (c) the right at any time to amend or supplement the responses; and (d) the right to move to quash or modify the Subpoena, or to otherwise seek appropriate relief with regard to the Subpoena.

12. Scantibodies incorporates each of the foregoing General Objections into the following Specific Responses and Objections as if fully set forth, and specifically reserves any and all objections to the Subpoena.

SPECIFIC RESPONSES AND OBJECTIONS

REQUEST NO. 1:

All documents and things relating to any agreements between Scantibodies and C&D and/or any other party relating to the manufacture of any C&D Test Kit, including but not limited to any such agreements themselves.

RESPONSE TO REQUEST NO. 1:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 2:

All documents and things relating to any obligation by Scantibodies to indemnify or otherwise reimburse or contribute to payment by C&D of any damages awarded to Abbott as a result of C&D's alleged infringement of the Patents-In-Suit.

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RESPONSE TO REQUEST NO. 2:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 3:

All documents and things relating to any obligation by C&D to indemnify or otherwise reimburse or contribute to payment by Scantibodies of any damages awarded to Abbott as a result of C&D's alleged infringement of the Patents-in-Suit.

RESPONSE TO REQUEST NO. 3:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 4:

All communications between Scantibodies and C&D relating to the decision to enter into, or to forgo, any agreement relating to the manufacture of any C&D Test Kit.

RESPONSE TO REQUEST NO. 4:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or

proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 5:

All communications between Scantibodies and C&D relating to the manufacture of any C&D Test Kit.

RESPONSE TO REQUEST NO. 5:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 6:

All documents relating to materials, ingredients or components used in the manufacture of any C&D Test Kit.

RESPONSE TO REQUEST NO. 6:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the

undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 7:

All documents relating to the manufacture by Scantibodies of any C&D Test Kit, including manufacturing processes, manufacturing specifications, raw materials specifications, and engineering drawings.

RESPONSE TO REQUEST NO. 7:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 8:

All documents relating to the design and development of any C&D Test Kit.

RESPONSE TO REQUEST NO. 8:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

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REQUEST NO. 9:

All documents and things relating to and/or referring to any antibody used in the C&D Test Kit including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

RESPONSE TO REQUEST NO. 9:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 10:

Samples of each of the antibodies used in the C&D test kits from 1999 to the present, including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

RESPONSE TO REQUEST NO. 10:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of materials that contain or are confidential or proprietary business information, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

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REQUEST NO. 11:

All tests, analyses, evaluations and/or documents referring to, relating to and/or performed with each of the antibodies used in the C&D Test Kits, including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

RESPONSE TO REQUEST NO. 11:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 12:

All documents and things relating to and/or referring to the binding capabilities of each of the antibodies used in the C&D Test Kits, including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

RESPONSE TO REQUEST NO. 12:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of materials that contain or are confidential or proprietary business information, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

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REQUEST NO. 13:

Samples of the streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

RESPONSE TO REQUEST NO. 13:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 14:

All documents and things relating to and/or referring to the streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

RESPONSE TO REQUEST NO. 14:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 15:

All tests, analysis, evaluations and/or documents referring to, relating to and/or performed with the streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

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RESPONSE TO REQUEST NO. 15:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 16:

All documents and things relating to and/or referring to the binding capabilities of the streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

RESPONSE TO REQUEST NO. 16:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 17:

All documents and things relating to the amount of each of the antibodies used in the C&D Test Kits, including but not limited to 11D6-2B10, CCF01, FSH132, 057-10036, HCG5F6, 2B2, B109, LH 26 and 5304.

RESPONSE TO REQUEST NO. 17:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or

proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

REQUEST NO. 18:

All documents and things relating to the amount of streptavidin, avidin and/or NeutrAvidin used in the C&D Test Kits.

RESPONSE TO REQUEST NO. 18:

Scantibodies objects to this Request on the grounds that it is overbroad, unduly burdensome, vague and ambiguous, seeks production of documents that contain confidential or proprietary business information, are protected by the attorney-client or work product protection, and are not relevant or reasonably calculated to lead to the discovery of admissible evidence. Unless and until Abbott is unable to obtain the requested documents from plaintiff Church & Dwight Co., Inc. through discovery in the underlying case, Scantibodies objects to being put to the undue burden and expense of locating and producing any non-privileged documents that might be responsive to this Request that it might have in its possession, custody or control.

DATED: May 16, 2008

LUCE, FORWARD, HAMILTON & SCRIPPS LLP

By: Edward P. Swan, Jr.
Edward Patrick Swan, Jr.
Attorneys for Scantibodies Laboratory, Inc.

101092466.1

CERTIFICATE OF SERVICE

Abbott Laboratories v. Church & Dwight Co., Inc.
U.S. District Court, Southern District of California
Case No. 07-CV-3428 (MFK) (Pending in the Northern District of Illinois)

I am employed with the law firm of Luce, Forward, Hamilton & Scripps LLP, whose address is 600 West Broadway, Suite 2600, San Diego, California 92101-3372. I am readily familiar with the business practices of this office for collection and processing of correspondence for mailing with the United States Postal Service; I am over the age of eighteen years, and am not a party to this action.

On May 20, 2008, I served the following:

NON-PARTY SCANTIBODIES LABORATORY, INC.'S RESPONSES AND OBJECTIONS TO PLAINTIFF ABBOTT LABORATORIES' SUBPOENA DUCES TECUM

on the below parties in this action by placing a true copy (copies) thereof in a separate envelope(s), addressed as shown, for collection and mailing on the below indicated day pursuant to the ordinary business practice of this office which is that correspondence for mailing is collected and deposited with the United States Postal Service on the same day in the ordinary course of business:

Stephanie McCallum, Esq.
Winston & Strawn LLP
35 W. Wacker Drive
Chicago, IL 60601
Attorneys for Abbott Laboratories

Baldo Vinti, Esq.
Proskauer Rose LLP
1585 Broadway
New York, NY 10036-8299
Attorneys for Church & Dwight Co., Inc.

I declare that I am employed in the office of a member of the bar of this court at whose direction the service was made.

Executed at San Diego, California on May 20, 2008.

Dorenne Charles

101094881.1

Warner, Kevin E.

From: Warner, Kevin E.
Sent: Monday, June 23, 2008 5:00 PM
To: 'pswan@luce.com'
Cc: McCallum, Stephanie S.
Subject: Abbott Labs. v. Church & Dwight - subpoena

Pat -
I am writing to follow up on our previous conversation and my voice mail from last week regarding Scantibodies' response to the subpoena served by Abbott in this matter. With respect to the requested documents, please promptly provide a date certain prior to July 3 on which Abbott can expect to receive all responsive documents from Scantibodies. With respect to the subpoenaed deposition, please promptly identify which person or persons Scantibodies will designate to testify, and provide dates available for a deposition to take place prior to July 16.

Feel free to contact me if you have any questions.

Regards,
Kevin

Kevin E. Warner
Associate

Winston & Strawn LLP
35 West Wacker Drive
Chicago, IL 60601-9703
T: +1 (312) 558-5852
F: +1 (312) 558-5700

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WINSTON
&STRAWN
LLP

Warner, Kevin E.

From: Warner, Kevin E.
Sent: Monday, July 21, 2008 10:31 PM
To: 'pswan@luce.com'
Cc: McCallum, Stephanie S.; Perkins, Ray; Warner, Kevin E.
Subject: RE: Abbott Labs. v. Church & Dwight - subpoena

Pat-
I have not received any response to the below e-mail, or to my voice mail of last week regarding the same.

Please immediately identify the witness(es) Scantibodies will present for deposition, and provide all available dates in August for that deposition. We request that information by Thursday, July 24. Please also immediately produce all documents we have previously discussed, including but not limited to all documents related to testing and certification of the reagents used in the accused products manufactured for Church & Dwight. We ask that you provide those documents to us no later than Friday, July 25th. If you do not intend to meet those deadlines, please advise us of your availability on Thursday, July 24, for a meet and confer conference regarding these issues.

We look forward to your response, and please do not hesitate to contact me with any questions.

Regards,
Kevin

Kevin E. Warner
Associate

Winston & Strawn LLP
35 West Wacker Drive
Chicago, IL 60601-9703
T: +1 (312) 558-5600
F: +1 (312) 558-5700

email | www.winston.com

WINSTON
&STRAWN
LLP

From: Warner, Kevin E.
Sent: Monday, June 23, 2008 5:00 PM
To: 'pswan@luce.com'
Cc: McCallum, Stephanie S.
Subject: Abbott Labs. v. Church & Dwight - subpoena

Pat -
I am writing to follow up on our previous conversation and my voice mail from last week regarding Scantibodies' response to the subpoena served by Abbott in this matter. With respect to the requested documents, please promptly provide a date certain prior to July 3 on which Abbott can expect to receive all responsive documents from Scantibodies. With respect to the

subpoenaed deposition, please promptly identify which person or persons Scantibodies will designate to testify, and provide dates available for a deposition to take place prior to July 16.

Feel free to contact me if you have any questions.

Regards,
Kevin

Kevin E. Warner
Associate

Winston & Strawn LLP
35 West Wacker Drive
Chicago, IL 60601-9703
T: +1 (312) 558-5852
F: +1 (312) 558-5700

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WINSTON
& STRAWN
LLP

Warner, Kevin E.

From: Swan, Pat [pswan@LUCE.com]
Sent: Thursday, July 24, 2008 12:44 PM
To: Warner, Kevin E.
Cc: Swan, Pat
Subject: RE: Abbott Labs. v. Church & Dwight - subpoena

Dear Kevin,

I called and left you a message to call me back.

As I understand it, the only documents you are seeking from SLI are those described in no. 11 of the List of Documents attached to the subpoena. Unfortunately, there is no time limitation in no. 11, making it overbroad, burdensome and oppressive. We will produce responsive documents, but we need to have an agreed-upon time limitation. The description in no. 11 is also overbroad. Based on our communications, I understand that the request seeks documents relating to the testing of the antibodies. Your e-mail below also mentions certifications, which is not in no. 11. I would appreciate it if you would clarify what you are requesting.

Thanks, Pat

From: Warner, Kevin E. [mailto:KWarner@winston.com]
Sent: Monday, July 21, 2008 8:31 PM
To: Swan, Pat
Cc: McCallum, Stephanie S.; Perkins, Ray; Warner, Kevin E.
Subject: RE: Abbott Labs. v. Church & Dwight - subpoena

Pat-
I have not received any response to the below e-mail, or to my voice mail of last week regarding the same.

Please immediately identify the witness(es) Scantibodies will present for deposition, and provide all available dates in August for that deposition. We request that information by Thursday, July 24. Please also immediately produce all documents we have previously discussed, including but not limited to all documents related to testing and certification of the reagents used in the accused products manufactured for Church & Dwight. We ask that you provide those documents to us no later than Friday, July 25th. If you do not intend to meet those deadlines, please advise us of your availability on Thursday, July 24, for a meet and confer conference regarding these issues.

We look forward to your response, and please do not hesitate to contact me with any questions.

Regards,
Kevin

Kevin E. Warner

Associate

Winston & Strawn LLP
35 West Wacker Drive
Chicago, IL 60601-9703
T: +1 (312) 558-5600
F: +1 (312) 558-5700

email | www.winston.com

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&STRAWN
LLP

From: Warner, Kevin E.
Sent: Monday, June 23, 2008 5:00 PM
To: 'pswan@luce.com'
Cc: McCallum, Stephanie S.
Subject: Abbott Labs. v. Church & Dwight - subpoena

Pat -
I am writing to follow up on our previous conversation and my voice mail from last week regarding Scantibodies' response to the subpoena served by Abbott in this matter. With respect to the requested documents, please promptly provide a date certain prior to July 3 on which Abbott can expect to receive all responsive documents from Scantibodies. With respect to the subpoenaed deposition, please promptly identify which person or persons Scantibodies will designate to testify, and provide dates available for a deposition to take place prior to July 16.

Feel free to contact me if you have any questions.

Regards,
Kevin

Kevin E. Warner
Associate

Winston & Strawn LLP
35 West Wacker Drive
Chicago, IL 60601-9703
T: +1 (312) 558-5852
F: +1 (312) 558-5700

bio | vcard | email | www.winston.com

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http://www.luce.com


Please consider the environment before printing this email.

Warner, Kevin E.

From: Warner, Kevin E.
Sent: Monday, August 04, 2008 11:20 PM
To: 'Swan, Pat'
Cc: McCallum, Stephanie S.
Subject: RE: Abbott Laboratories et al. v. Church & Dwight, 07-3428

Pat -
Thank you for agreeing to accept service of the subpoena. We consider the subpoena as having been served today, August 4, 2008.

I have tried on several occasions to call you to discuss your e-mail of July 24 to clarify Abbott's requests, but have been unable to reach you. In order to facilitate SLI's production, please note the following. First, during our last phone conversation to discuss SLI's production in response to the subpoena, I highlighted the category of documents exemplified by Request No. 11 as a category of documents that may not have been produced by Church & Dwight in this litigation. Such documents would thus not be subject to any objection by SLI that further production would be duplicative. Abbott is not, however, withdrawing any or all of its other document requests. To the extent SLI has documents responsive to those categories that have not been produced in this litigation by Church & Dwight, we request that you produce those documents as well.

Abbott believes Request No. 11 is proper in terms of the scope of its subject matter and is not objectionable because, as you claim, there is no time limitation. Documents concerning the the antibodies used in any accused C&D Test Kit are relevant, regardless of the date of their creation. For instance, documents concerning the properties and characteristics of the antibodies used in the accused Test Kits are relevant to the extent they were created during the period of SLI's relationship with Church & Dwight. Such documents that relate to the same antibodies and/or cell lines are not irrelevant simply because they may pre-date that relationship.

With respect to my comment about "certifications," Abbott is asking for any documents such as certificates or specifications that report any properties or characteristics of the subject antibodies. Such certificates would be, for instance, formal documentation of the "tests, analyses [or] evaluations" that are called for in Request No. 11.

By this Thursday, please provide a date certain on or before August 15 on which these documents will be produced. We believe that date is more than reasonable, given that SLI has had this subpoena since April 2008.

We have also not received any proposed dates regarding the Rule 30(b)(6) deposition of SLI. By Thursday, please also provide all available dates in August for the Rule 30(b)(6) deposition of SLI.

I am traveling for the next few days. If you have any further questions about the subpoena, please contact me at 773-727-1988.

Regards,
Kevin

From: Swan, Pat [mailto:pswan@LUCE.com]
Sent: Monday, August 04, 2008 10:27 AM
To: Warner, Kevin E.
Cc: McCallum, Stephanie S.; Swan, Pat
Subject: RE: Abbott Laboratories et al. v. Church & Dwight, 07-3428

Dear Kevin,

I can accept service of the new subpoena to SLI.

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"I called and left you a message to call me back.

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I would appreciate it if you would let me know.

Yours, Pat

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Sent: Friday, August 01, 2008 4:20 PM
To: Swan, Pat
Cc: McCallum, Stephanie S.; Warner, Kevin E.
Subject: Abbott Laboratories et al. v. Church & Dwight, 07-3428

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Please let me know by close of business on Monday, August 4, whether you can and will accept service of the attached subpoena to Scantibodies Laboratory. We will have the subpoena served directly if we do not hear from you by then.

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Warner, Kevin E.

From: Swan, Pat [pswan@LUCE.com]
Sent: Sunday, August 10, 2008 11:47 PM
To: Warner, Kevin E.
Cc: Swan, Pat
Subject: Abbott Laboratories et al. v. Church & Dwight, 07-3428

Dear Kevin,

I am disappointed in your message below. Despite our earlier conversation and agreement that you were now only seeking documents pursuant to Request No. 11, you are now attempting to renege. That is unacceptable.

As to Request No. 11, the lack of a time limitation makes it unreasonable. Further, it ignores the fact that the product has a two-year shelf life. We will produce responsive, non-privileged documents going back two years. We hope to have these documents copied by August 25th.

Scantibodies' Rule 30(b)(6) witness on testing will be Jerry Sun. I assume you will want to depose him after you receive the documents. Please let me know what dates are available for you after August 25th.

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email | www.winston.com

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Any tax advice contained in this email was not intended to be used, and cannot be used, by you (or any other taxpayer) to avoid penalties under the Internal Revenue Code of 1986, as amended.

****

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http://www.luce.com


Please consider the environment before printing this email.

Warner, Kevin E.

From: Warner, Kevin E.
Sent: Monday, August 11, 2008 8:42 AM
To: 'pswan@luce.com'
Cc: Warner, Kevin E.; Perkins, Ray
Subject: RE: Abbott Laboratories et al. v. Church & Dwight, 07-3428

Pat,
You have mischaracterized our earlier conversation about Abbott's subpoena to SLI. I am sure you recall that there was no specific mention whatsoever of "Topic 11" during any of our previous phone conversations, and I never agreed to narrow the subpoena to any particular document categories and forego others. I am not attempting to "renege" on any agreement, and I stand by my representation below of our previous conversations.

Further, your objection regarding the time limit of Topic No. 11 is untenable and does not appear to be made in good faith. The "shelf-life" of any of the relevant antibodies is of course irrelevant to what documents might be responsive. A document that characterizes an antibody prior to the expiration of its "shelf-life" does not become irrelevant or of less importance at any time thereafter simply because the antibody itself may no longer be active. In any event, we will not further debate this issue and will seek the Court's assistance in enforcing the subpoena.

As for the deposition of Jerry Sun, we are available any day during the weeks of September 1 or September 8. We need to take the deposition after Abbott's inspection of Scantibodies' premises, so we request that you immediately provide dates for both during those weeks.

Regards,
Kevin

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****
Any tax advice contained in this email was not intended to be used, and cannot be used, by you (or any other taxpayer) to avoid penalties under the Internal Revenue Code of 1986, as amended.

****

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Please consider the environment before printing this email.

AO88 (Rev. 12/07) Subpoena in a Civil Case

Issued by the

UNITED STATES DISTRICT COURT

Southern District of California

Abbott Laboratories and SurModics, Inc.

V.

Church & Dwight Co., Inc.

SUBPOENA IN A CIVIL CASE

Case Number:[1] 1:07-cv-03428 (MFK) (N.D. Ill.)

TO: Scantibodies Laboratory, Inc.
9336 Abraham Way
San Diego, CA 92121-2904

☐ YOU ARE COMMANDED to appear in the United States District court at the place, date, and time specified below to testify in the above case.

PLACE OF TESTIMONY	COURTROOM
	DATE AND TIME

☐ YOU ARE COMMANDED to appear at the place, date, and time specified below to testify at the taking of a deposition in the above case.

PLACE OF DEPOSITION	DATE AND TIME

☐ YOU ARE COMMANDED to produce and permit inspection and copying of the following documents or objects at the place, date, and time specified below (list documents or objects):

See Schedule A, attached.

PLACE	DATE AND TIME

☑ YOU ARE COMMANDED to permit inspection of the following premises at the date and time specified below.

PREMISES	DATE AND TIME
See Schedule A, attached	8/22/2008 9:00 am

Any organization not a party to this suit that is subpoenaed for the taking of a deposition shall designate one or more officers, directors, or managing agents, or other persons who consent to testify on its behalf, and may set forth, for each person designated, the matters on which the person will testify. Federal Rule of Civil Procedure 30(b)(6).

ISSUING OFFICER'S SIGNATURE AND TITLE (INDICATE IF ATTORNEY FOR PLAINTIFF OR DEFENDANT)	DATE
Attorney for Plaintiffs Abbott Laboratories and SurModics, Inc.	8/1/2008

ISSUING OFFICER'S NAME, ADDRESS AND PHONE NUMBER

Kevin E. Warner, Esq., Winston & Strawn LLP, 35 West Wacker Dr., Chicago, IL 60601
(312) 558-5600

(See Federal Rule of Civil Procedure 45 (c), (d), and (e), on next page)

[1] If action is pending in district other than district of issuance, state district under case number.

PROOF OF SERVICE

DATE PLACE

SERVED

SERVED ON (PRINT NAME) MANNER OF SERVICE

SERVED BY (PRINT NAME) TITLE

DECLARATION OF SERVER

I declare under penalty of perjury under the laws of the United States of America that the foregoing information contained in the Proof of Service is true and correct.

Executed on _____
DATE

_____
SIGNATURE OF SERVER

_____
ADDRESS OF SERVER

Federal Rule of Civil Procedure 45 (c), (d), and (e), as amended on December 1, 2007:

(c) PROTECTING A PERSON SUBJECT TO A SUBPOENA.

(1) Avoiding Undue Burden or Expense; Sanctions. A party or attorney responsible for issuing and serving a subpoena must take reasonable steps to avoid imposing undue burden or expense on a person subject to the subpoena. The issuing court must enforce this duty and impose an appropriate sanction — which may include lost earnings and reasonable attorney's fees — on a party or attorney who fails to comply.

(2) Command to Produce Materials or Permit Inspection.

(A) Appearance Not Required. A person commanded to produce documents, electronically stored information, or tangible things, or to permit the inspection of premises, need not appear in person at the place of production or inspection unless also commanded to appear for a deposition, hearing, or trial.

(B) Objections. A person commanded to produce documents or tangible things or to permit inspection may serve on the party or attorney designated in the subpoena a written objection to inspecting, copying, testing or sampling any or all of the materials or to inspecting the premises — or to producing electronically stored information in the form or forms requested. The objection must be served before the earlier of the time specified for compliance or 14 days after the subpoena is served. If an objection is made, the following rules apply:

(i) At any time, on notice to the commanded person, the serving party may move the issuing court for an order compelling production or inspection.

(ii) These acts may be required only as directed in the order, and the order must protect a person who is neither a party nor a party's officer from significant expense resulting from compliance.

(3) Quashing or Modifying a Subpoena.

(A) When Required. On timely motion, the issuing court must quash or modify a subpoena that:

(i) fails to allow a reasonable time to comply;

(ii) requires a person who is neither a party nor a party's officer to travel more than 100 miles from where that person resides, is employed, or regularly transacts business in person — except that, subject to Rule 45(c)(3)(B)(iii), the person may be commanded to attend a trial by traveling from any such place within the state where the trial is held;

(iii) requires disclosure of privileged or other protected matter, if no exception or waiver applies; or

(iv) subjects a person to undue burden.

(B) When Permitted. To protect a person subject to or affected by a subpoena, the issuing court may, on motion, quash or modify the subpoena if it requires:

(i) disclosing a trade secret or other confidential research, development, or commercial information;

(ii) disclosing an unretained expert's opinion or information that does not describe specific occurrences in dispute and results from the expert's study that was not requested by a party; or

(iii) a person who is neither a party nor a party's officer to incur substantial expense to travel more than 100 miles to attend trial

(C) Specifying Conditions as an Alternative. In the circumstances described in Rule 45(c)(3)(B), the court may, instead of quashing or modifying a subpoena, order appearance or production under specified conditions if the serving party:

(i) shows a substantial need for the testimony or material that cannot be otherwise met without undue hardship; and

(ii) ensures that the subpoenaed person will be reasonably compensated.

(d) DUTIES IN RESPONDING TO A SUBPOENA.

(1) Producing Documents or Electronically Stored Information. These procedures apply to producing documents or electronically stored information:

(A) Documents. A person responding to a subpoena to produce documents must produce them as they are kept in the ordinary course of business or must organize and label them to correspond to the categories in the demand.

(B) Form for Producing Electronically Stored Information Not Specified. If a subpoena does not specify a form for producing electronically stored information, the person responding must produce it in a form or forms in which it is ordinarily maintained or in a reasonably usable form or forms.

(C) Electronically Stored Information Produced in Only One Form. The person responding need not produce the same electronically stored information in more than one form.

(D) Inaccessible Electronically Stored Information. The person responding need not provide discovery of electronically stored information from sources that the person identifies as not reasonably accessible because of undue burden or cost. On motion to compel discovery or for a protective order, the person responding must show that the information is not reasonably accessible because of undue burden or cost. If that showing is made, the court may nonetheless order discovery from such sources if the requesting party shows good cause, considering the limitations of Rule 26(b)(2)(C). The court may specify conditions for the discovery.

(2) Claiming Privilege or Protection.

(A) Information Withheld. A person withholding subpoenaed information under a claim that it is privileged or subject to protection as trial-preparation material must:

(i) expressly make the claim; and

(ii) describe the nature of the withheld documents, communications, or tangible things in a manner that, without revealing information itself privileged or protected, will enable the parties to assess the claim.

(B) Information Produced. If information produced in response to a subpoena is subject to a claim of privilege or of protection as trial-preparation material, the person making the claim may notify any party that received the information of the claim and the basis for it. After being notified, a party must promptly return, sequester, or destroy the specified information and any copies it has; must not use or disclose the information until the claim is resolved; must take reasonable steps to retrieve the information if the party disclosed it before being notified; and may promptly present the information to the court under seal for a determination of the claim. The person who produced the information must preserve the information until the claim is resolved.

(e) CONTEMPT.

The issuing court may hold in contempt a person who, having been served, fails without adequate excuse to obey the subpoena. A nonparty's failure to obey must be excused if the subpoena purports to require the nonparty to attend or produce at a place outside the limits of Rule 45(c)(3)(A)(ii).

SCHEDULE A

UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF ILLINOIS
EASTERN DIVISION

ABBOTT LABORATORIES and SURMODICS, INC., (involuntary plaintiff), Plaintiffs, v. CHURCH & DWIGHT CO., INC., Defendant.	Honorable Matthew Kennelly, U.S.D.J. Case No. 07 CV 3428

PLAINTIFF ABBOTT LABORATORIES' RULE 34 REQUEST TO THIRD PARTY SCANTIBODIES TO PERMIT ENTRY AND INSPECTION OF SCANTIBODIES' PREMISES

Pursuant to Rules 34 and 45 of the Federal Rules of Civil Procedure, Plaintiff Abbott Laboratories ("Abbott") requests that third-party Scantibodies Laboratory, Inc. ("Scantibodies") permit Abbott to enter Scantibodies' (a) production plant or facilities; (b) manufacturing plant or facilities; (c) assembly plant or facilities; and/or (d) quality control and assurance plant or facilities, wherever located but including at least at 9336 Abraham Way, Santee, California 92071, for the purpose of inspecting, photographing and videotaping premises, objects, product, equipment and operations that concern the production, manufacture and/or assembly of any test for the diagnosis of pregnancy or ovulation for or on behalf of Church & Dwight Co., Inc., or any component part thereof ("C&D Test Kits").

Inspecting, photographing and/or videotaping of the designated plant, facilities or other premises, shall commence at 9:00 a.m. on August 22, 2008, and shall continue until completed. Inspection of the designated plant, facilities and/or other premises shall be during an actual production run or runs of commercial batches of C&D Test Kits for the detection of pregnancy and ovulation. The requested inspection may be conducted by counsel for Abbott, a videographer, photographer and one or more experts.

Dated: August 1, 2008

Respectfully Submitted,

ABBOTT LABORATORIES

By: _____

One of its Attorneys

George C. Lombardi
Raymond Perkins
Stephanie McCallum
Kevin E. Warner
WINSTON & STRAWN LLP
35 West Wacker Drive
Chicago, IL 60601
(312) 558-5600

Attorneys for Plaintiff Abbott Laboratories

CERTIFICATE OF SERVICE

The undersigned, an attorney, certifies that a true and correct copy of the following document:

PLAINTIFF ABBOTT LABORATORIES' RULE 34 REQUEST TO THIRD PARTY SCANTIBODIES TO PERMIT ENTRY AND INSPECTION OF SCANTIBODIES' PREMISES

was served on August 1, 2008, by the method indicated:

BY EMAIL AND U.S. MAIL:
Anastasia Heffner
Glen Belvis
BRINKS, HOFER, GILSON & LIONE
NBC Tower, Suite 3600
455 North Cityfront Plaza Drive
Chicago, IL 60611-5599

BY EMAIL AND U.S. MAIL
James H. Shalek
Baldassare Vinti
John Stellabotte
Steven Hollinstat
PROSKAUER ROSE LLP
1585 Broadway
New York, NY 10036-8299
212.969.2900 (fax)

BY EMAIL AND U.S. MAIL
Cyrus A. Morton
Trevor J. Foster
2800 LaSalle Plaza
800 LaSalle Avenue
Minneapolis, MN 55402

/s/ Kevin E. Warner
Kevin E. Warner

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

CERTIFICATE OF SERVICE

Abbott Laboratories v. Scantibodies Laboratory, Inc.

I, Stephen R. Smerek, an attorney at Winston & Strawn LLP, of the office located at 333 South Grand Avenue, 38th Floor, Los Angeles, CA 90071-1543, hereby certify under penalty of perjury that on August 19, 2008, that I caused to be served the following documents entitled:

1. SUMMONS

2. ABBOTT LABORATORIES' NOTICE OF MOTION AND MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES PURSUANT TO RULE 45(C)(2)(B); MEMORANDUM OF POINTS AND AUTHORITIES AND DECLARATION OF KEVIN E. WARNER IN SUPPORT THEREOF

3. PROPOSED ORDER COMPELLING COMPLIANCE WITH SUBPOENA ON SCANTIBODIES LABORATORY, INC.

4. ABBOTT LABORATORIES' CORPORATE DISCLOSURE STATEMENT

Said documents were served as indicated below, upon all interested parties at the following addresses:

BY HAND:

Allen Garrett, Registered Agent
SCANTIBODIES LABORATORY, INC.
9336 Abraham Way
Santee, CA 92071

BY ELECTRONIC MAIL AND FEDEX PRIORITY OVERNIGHT MAIL:

Edward Patrick Swan, Jr.
LUCE, FORWARD, HAMILTON & SCRIPPS LLP
600 West Broadway, Suite 2600
San Diego, CA 92101-3372
pswan@luce.com

Attorneys for Scantibodies Laboratory, Inc.

BY ELECTRONIC AND U.S. MAIL:

Anastasia Heffner
Glen Belvis
BRINKS, HOFER, GILSON & LIONE

NBC Tower, Suite 3600
455 North Cityfront Plaza Drive
Chicago, IL 60611-5599
aheffener@usebrinks.com
gbelvis@usebrinks.com

James H. Shalek
Baldassare Vinti
John Stellabotte
Steven Holinstat
PROSKAUER ROSE LLP
1585 Broadway
New York, NY 10036-8299
jshalek@proskauer.com
bvinti@proskauer.com
jstellabotte@proskauer.com
sholinstat@proskauer.com

Cyrus A. Morton
Trevor J. Foster
ROBINS, KAPLAN, MILLER & CIRESI
2800 LaSalle Plaza
800 LaSalle Avenue
Minneapolis, MN 55402
cmorton@rkmc.com
tjfoster@rkmc.com

Attorneys for Church & Dwight Co., Inc.

DATED: AUGUST 19, 2008

Stephen R. Smerek

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

EXHIBIT 2

ORIGINAL

JS 44 (Rev. 12/07)

CIVIL COVER SHEET

The JS 44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM.)

FILED
2008 AUG 19 PM 4:34
CLERK US DISTRICT COURT
SOUTHERN DISTRICT OF CALIFORNIA
BY DEPUTY

'08 CV 1525 H BLM

I. (a) PLAINTIFFS
ABBOTT LABORATORIES

DEFENDANTS
SCANTIBODIES LABORATORY, INC.

(b) County of Residence of First Listed Plaintiff Lake County, IL
(EXCEPT IN U.S. PLAINTIFF CASES)

County of Residence of First Listed Defendant San Diego County, CA
(IN U.S. PLAINTIFF CASES ONLY)
NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE LAND INVOLVED.

(c) Attorney's (Firm Name, Address, and Telephone Number)
Stephen R. Smerek, WINSTON & STRAWN LLP; 333 South Grand Avenue, Fl. 38, Los Angeles, CA 90071; T: 213-615-1700

Attorneys (If Known)
Edward P. Swan, Jr., LUCE,FORWARD,HAMILTON&SCRIPPS LLP; 600 W. Broadway, San Diego, CA 92101; T:619-699-2418

II. BASIS OF JURISDICTION (Place an "X" in One Box Only)

- ☐ 1 U.S. Government Plaintiff
- ☒ 3 Federal Question (U.S. Government Not a Party)
- ☐ 2 U.S. Government Defendant
- ☐ 4 Diversity (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES (Place an "X" in One Box for Plaintiff and One Box for Defendant)
(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	☐ 1	☐ 1	Incorporated or Principal Place of Business In This State	☐ 4	☐ 4
Citizen of Another State	☐ 2	☐ 2	Incorporated and Principal Place of Business In Another State	☐ 5	☐ 5
Citizen or Subject of a Foreign Country	☐ 3	☐ 3	Foreign Nation	☐ 6	☐ 6

IV. NATURE OF SUIT (Place an "X" in One Box Only)

CONTRACT	TORTS		FORFEITURE/PENALTY	BANKRUPTCY	OTHER STATUTES
☐ 110 Insurance	PERSONAL INJURY	PERSONAL INJURY	☐ 610 Agriculture	☐ 422 Appeal 28 USC 158	☐ 400 State Reapportionment
☐ 120 Marine	☐ 310 Airplane	☐ 362 Personal Injury - Med. Malpractice	☐ 620 Other Food & Drug	☐ 423 Withdrawal 28 USC 157	☐ 410 Antitrust
☐ 130 Miller Act	☐ 315 Airplane Product Liability	☐ 365 Personal Injury - Product Liability	☐ 625 Drug Related Seizure of Property 21 USC 881	PROPERTY RIGHTS	☐ 430 Banks and Banking
☐ 140 Negotiable Instrument	☐ 320 Assault, Libel & Slander	☐ 368 Asbestos Personal Injury Product Liability	☐ 630 Liquor Laws	☐ 820 Copyrights	☐ 450 Commerce
☐ 150 Recovery of Overpayment & Enforcement of Judgment	☐ 330 Federal Employers' Liability	PERSONAL PROPERTY	☐ 640 R.R. & Truck	☐ 830 Patent	☐ 460 Deportation
☐ 151 Medicare Act	☐ 340 Marine	☐ 370 Other Fraud	☐ 650 Airline Regs	☐ 840 Trademark	☐ 470 Racketeer Influenced and Corrupt Organizations
☐ 152 Recovery of Defaulted Student Loans (Excl. Veterans)	☐ 345 Marine Product Liability	☐ 371 Truth in Lending	☐ 660 Occupational Safety/Health		☐ 480 Consumer Credit
☐ 153 Recovery of Overpayment of Veteran's Benefits	☐ 350 Motor Vehicle	☐ 380 Other Personal Property Damage	☐ 690 Other		☐ 490 Cable/Sat TV
☐ 160 Stockholders' Suits	☐ 355 Motor Vehicle Product Liability	☐ 385 Property Damage Product Liability	LABOR	SOCIAL SECURITY	☐ 810 Selective Service
☐ 190 Other Contract	☐ 360 Other Personal Injury		☐ 710 Fair Labor Standards Act	☐ 861 HIA (1395ff)	☐ 850 Securities/Commodities/ Exchange
☐ 195 Contract Product Liability			☐ 720 Labor/Mgmt. Relations	☐ 862 Black Lung (923)	☐ 875 Customer Challenge 12 USC 3410
☐ 196 Franchise			☐ 730 Labor/Mgmt.Reporting & Disclosure Act	☐ 863 DIWC/DIWW (405(g))	☒ 890 Other Statutory Actions
REAL PROPERTY	CIVIL RIGHTS	PRISONER PETITIONS	☐ 740 Railway Labor Act	☐ 864 SSID Title XVI	☐ 891 Agricultural Acts
☐ 210 Land Condemnation	☐ 441 Voting	☐ 510 Motions to Vacate Sentence	☐ 790 Other Labor Litigation	☐ 865 RSI (405(g))	☐ 892 Economic Stabilization Act
☐ 220 Foreclosure	☐ 442 Employment	Habeas Corpus:	☐ 791 Empl. Ret. Inc. Security Act	FEDERAL TAX SUITS	☐ 893 Environmental Matters
☐ 230 Rent Lease & Ejectment	☐ 443 Housing/ Accommodations	☐ 530 General	IMMIGRATION	☐ 870 Taxes (U.S. Plaintiff or Defendant)	☐ 894 Energy Allocation Act
☐ 240 Torts to Land	☐ 444 Welfare	☐ 535 Death Penalty	☐ 462 Naturalization Application	☐ 871 IRS—Third Party 26 USC 7609	☐ 895 Freedom of Information Act
☐ 245 Tort Product Liability	☐ 445 Amer. w/Disabilities - Employment	☐ 540 Mandamus & Other	☐ 463 Habeas Corpus - Alien Detainee		☐ 900Appeal of Fee Determination Under Equal Access to Justice
☐ 290 All Other Real Property	☐ 446 Amer. w/Disabilities - Other	☐ 550 Civil Rights	☐ 465 Other Immigration Actions		☐ 950 Constitutionality of State Statutes
	☐ 440 Other Civil Rights	☐ 555 Prison Condition			

V. ORIGIN (Place an "X" in One Box Only)

☐ 1 Original Proceeding ☐ 2 Removed from State Court ☐ 3 Remanded from Appellate Court ☐ 4 Reinstated or Reopened ☐ 5 Transferred from another district (specify) ☒ 6 Multidistrict Litigation ☐ 7 Appeal to District Judge from Magistrate Judgment

VI. CAUSE OF ACTION
Cite the U.S. Civil Statute under which you are filing (Do not cite jurisdictional statutes unless diversity):
Fed. R. Civ. P. 45(c)
Brief description of cause:
Non-compliance with third party subpoena

VII. REQUESTED IN COMPLAINT:
☐ CHECK IF THIS IS A CLASS ACTION UNDER F.R.C.P. 23
DEMAND $
CHECK YES only if demanded in complaint:
JURY DEMAND: ☐ Yes ☒ No

VIII. RELATED CASE(S) IF ANY (See instructions):
JUDGE Judge Matthew F. Kennelly DOCKET NUMBER 07-CV-3428 (MFK) (N.D.Ill.)

DATE
08/18/2008

SIGNATURE OF ATTORNEY OF RECORD

FOR OFFICE USE ONLY

RECEIPT # AMOUNT APPLYING IFP JUDGE MAG. JUDGE

FILED

2008 AUG 19 PM 4:34

CLERK, U.S. DISTRICT COURT
SOUTHERN DISTRICT OF CALIFORNIA

BY _____ DEPUTY

Stephen R. Smerek (SBN [illegible])
WINSTON & STRAWN LLP
333 South Grand Avenue, 38th Floor
Los Angeles, CA 90071-1543
Telephone: 213-615-1700
Facsimile: 213-615-1750
Email: ssmerek@winston.com

George C. Lombardi
Raymond C. Perkins
Stephanie S. McCallum
Kevin E. Warner
WINSTON & STRAWN LLP
35 West Wacker Drive
Chicago, IL 60601
Telephone: 312-558-5600
Facsimile: 312-558-5700
Email: glombardi@winston.com

Attorneys for Plaintiff
ABBOTT LABORATORIES

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

08 CV 1525 H BLM

ABBOTT LABORATORIES,

Plaintiff,

v.

SCANTIBODIES LABORATORY, INC.,

Defendant.

Misc. Action No.

Case No. 07 CV 3428 (MFK)
Pending in the Northern District of Illinois

ABBOTT LABORATORIES' NOTICE OF MOTION AND MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES LABORATORY, INC., PURSUANT TO RULE 45(C)(2)(B); MEMORANDUM OF POINTS AND AUTHORITIES AND DECLARATION OF KEVIN E. WARNER IN SUPPORT THEREOF

Date: TBD
Time: TBD
Department: TBD

Stephen R. Smerek (SBN: 208343)
WINSTON & STRAWN LLP
333 South Grand Avenue, 38th Floor
Los Angeles, CA 90071-1543
Telephone: 213-615-1700
Facsimile: 213-615-1750
Email: ssmerek@winston.com

George C. Lombardi
Raymond C. Perkins
Stephanie S. McCallum
Kevin E. Warner
WINSTON & STRAWN LLP
35 West Wacker Drive
Chicago, IL 60601
Telephone: 312-558-5600
Facsimile: 312-558-5700
Email: glombardi@winston.com

Attorneys for Plaintiff
ABBOTT LABORATORIES

Winston & Strawn LLP
333 South Grand Avenue
Los Angeles, CA 90071-1543

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

ABBOTT LABORATORIES,

Plaintiff,

v.

SCANTIBODIES LABORATORY, INC.,

Defendant.

Misc. Action No. '08 CV 1525 H BLM

Case No. 07 CV 3428 (MFK)
Pending in the Northern District of Illinois

[PROPOSED] ORDER GRANTING ABBOTT LABORATORIES' MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES LABORATORY, INC. PURSUANT TO RULE 45(C)(2)(B)

Date: TBD
Time: TBD
Department: TBD

Stephen R. Smerek (SBN: 208343)
WINSTON & STRAWN LLP
333 South Grand Avenue, 38th Floor
Los Angeles, CA 90071-1543
Telephone: 213-615-1700
Facsimile: 213-615-1750
Email: ssmerek@winston.com

George C. Lombardi
Raymond C. Perkins
Stephanie S. McCallum
Kevin E. Warner
WINSTON & STRAWN LLP
35 West Wacker Drive
Chicago, IL 60601
Telephone: 312-558-5600
Facsimile: 312-558-5700
Email: glombardi@winston.com

Attorneys for Plaintiff
ABBOTT LABORATORIES

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

ABBOTT LABORATORIES,

Plaintiff,

v.

SCANTIBODIES LABORATORY, INC.,

Defendant.

Misc. Action No. '08 CV 1525 H BLM

Case No. 07 CV 3428 (MFK)
Pending in the Northern District of Illinois

ABBOTT LABORATORIES' CORPORATE DISCLOSURE STATEMENT

WINSTON & STRAWN LLP (213) 615-1700 **Stephen R. Smerek, Esq. (SBN 208343)** **333 South Grand Avenue, 38th Floor** **Los Angeles, California 90071-1543** ATTORNEY FOR (*Name*): **Plaintiff ABBOTT LABORATORIES**				
UNITED STATES DISTRICT COURT **SOUTHERN DISTRICT OF CALIFORNIA**				
PLAINTIFF: **NORA GARIBOTTI** DEFENDANT: **JAMES E. McCORMICK III, et al.**				
PROOF OF SERVICE	HEARING DATE:	TIME:	DEPT/DIV.:	CASE NUMBER: **08 CV 1525 H BLM**

1. At the time of service I was at least 18 years of age and not a party to this action, and I served copies of the (*specify document(s)*):
SEE ATTACHED LIST

2. a. Party served: **Scantibodies Laboratory, Inc.**

b. Person served: **Allan Garrett, Agent for service of process**

c. Address: **9343 Wheatlands Road**
Santee, California 92071-2860

3. I served the party in item 2
a. **by personally delivering** the copies (1) on (*date*): **08/20/08**
(2) at (*time*): **01:41 p.m.**

4. Person serving (*name, address, and telephone No.*):

Craig Clemence
Ace Messenger and Attorney Service, Inc.
110 West C Street, Suite 805
San Diego, California 92101
(619) 235-8400

a. Fee for service: $

b. Registered California process server.
(1) Employee or independent contractor.
(2) Registration No.: **1424**
(3) County: **SAN DIEGO**

5. I declare under penalty of perjury under the laws of the State of California that the foregoing is true and correct.

Date: **August 22, 2008**

Craig Clemence
(*Signature*)

ATTACHED LIST OF DOCUMENTS

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF CALIFORNIA

ABBOTT LABORATORIES
v.
SCANTIBODIES LABORATORY, INC.

CASE NUMBER: 08 CV 1525 H BLM

1. SUMMONS;

2. CIVIL CASE COVER SHEET;

3. ABBOTT LABORATORIES' NOTICE OF MOTION AND MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES LABORATORY, INC., PURSUANT TO RULE 45(C)(2)(B); MEMORANDUM OF POINTS AND AUTHORITIES AND DECLARATION OF KEVIN E. WARNER IN SUPPORT THEREOF;

4. [PROPOSED] ORDER GRANTNG ABBOTT LABORATORIES' MOTION TO COMPEL COMPLIANCE WITH SUBPOENA ON THIRD PARTY SCANTIBODIES LABORATORY, INC., PURSUANT TO RULE 45(C)(2)(B);;

5. ABBOTT LABORATORIES' CORPORATE DISCLOSURE STATEMENT ;